

ABIOTIC STRESS TOLERANCE FROM THE TERTIARY GENE POOL OF COMMON
WHEAT

by

ANDREW JUSTIN GREEN

B.S., Agricultural Science, Truman State University, 2009
M.S., Crop and Soil Environmental Science, Virginia Polytechnic Institute and State University,
2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Abstract

Heat and drought stress are two of the most significant abiotic stresses limiting wheat production in the Great Plains and worldwide. Introgression of novel tolerance genes from wild relatives is a strategy which presents promise. This study examined both heat and drought tolerance from the tetraploid species *Aegilops geniculata* (U^sU^sM^sM^s). Additional screening for heat tolerance was conducted with the US genome species *Aegilops peregrina* (Hack) and *Aegilops kotschy* (Boiss). A comprehensive screening system for drought tolerance was also constructed to evaluate wheat and its wild relatives.

Previous reports suggested that *Ae. geniculata* accession TA2899 was moderately tolerant to heat stress. It had also previously been used to develop a full set of wheat-*Ae. geniculata* chromosome addition lines in a Chinese Spring background. To identify the chromosome(s) carrying the heat tolerance, all addition lines, as well as wheat check genotypes, were screened for post-anthesis heat tolerance in two growth chamber experiments. No chromosome addition lines were significantly different ($p < 0.05$) from Chinese Spring, and none were found to have superior performance to the positive check cultivars.

Forty-five accessions of *Ae. peregrina* and its close relative, *Ae. kotschy* were screened in a post-anthesis heat experiment. A follow-up experiment compared the genotypes in a split-plot temperature treatment with heat and optimal growth chambers. Many accessions were similar to the control genotypes for grain fill duration, and some exceeded the wheat controls for relative chlorophyll index values on Day 12 and Day 16. TA1889 and TA1904, both *Ae. peregrina* accessions originating from Israel, had a higher grain fill duration across experiments than the best wheat control, and warrant further investigation.

Previous reports suggested drought tolerance in *Ae. geniculata*. After preliminary screenings, six genotypes were selected for advanced screening and compared with three wheat cultivars. The advanced greenhouse screening system was conducted in 152cm tall PVC growth tubes. The experiment measured multiple plant responses, and had a datalogging system automatically collecting water content and matric potential of the growth media. Multiple accessions warranted further investigation, and showed potentially different modes of drought tolerance, with varying levels of stomatal resistance, biomass, and osmotic adjustment.

ABIOTIC STRESS TOLERANCE FROM THE TERTIARY GENE POOL OF COMMON
WHEAT

by

ANDREW JUSTIN GREEN

B.S., Agricultural Science, Truman State University, 2009
M.S., Crop and Soil Environmental Science, Virginia Polytechnic Institute and State University,
2011

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Allan K. Fritz

Abstract

Heat and drought stress are two of the most significant abiotic stresses limiting wheat production in the Great Plains and worldwide. Introgression of novel tolerance genes from wild relatives is a strategy which presents promise. This study examined both heat and drought tolerance from the tetraploid species *Aegilops geniculata* (U⁵U⁵M⁵M⁵). Additional screening for heat tolerance was conducted with the US genome species *Aegilops peregrina* (Hack) and *Aegilops kotschy* (Boiss). A comprehensive screening system for drought tolerance was also constructed to evaluate wheat and its wild relatives.

Previous reports suggested that *Ae. geniculata* accession TA2899 was moderately tolerant to heat stress. It had also previously been used to develop a full set of wheat-*Ae. geniculata* chromosome addition lines in a Chinese Spring background. To identify the chromosome(s) carrying the heat tolerance, all addition lines, as well as wheat check genotypes, were screened for post-anthesis heat tolerance in two growth chamber experiments. No chromosome addition lines were significantly different ($p < 0.05$) from Chinese Spring, and none were found to have superior performance to the positive check cultivars.

All accessions of *Ae. peregrina* and its close relative, *Ae. kotschy* were screened in a post-anthesis heat experiment. A follow-up experiment compared the genotypes in a split-plot temperature treatment with heat and optimal growth chambers. Many accessions were similar to the control genotypes for grain fill duration, and some exceeded the wheat controls for relative chlorophyll index values on Day 12 and Day 16. TA1889 and TA1904, both *Ae. peregrina* accessions originating from Israel, had a higher grain fill duration across experiments than the best wheat control, and warrant further investigation.

Previous reports suggested drought tolerance in *Ae. geniculata*. After preliminary screenings, six genotypes were selected for advanced screening and compared with three wheat cultivars. The advanced greenhouse screening system was conducted in 152cm tall PVC growth tubes. The experiment measured multiple plant responses, and had a datalogging system automatically collecting water content and matric potential of the growth media. Multiple accessions warranted further investigation, and showed potentially different modes of drought tolerance, with varying levels of stomatal resistance, biomass, and osmotic adjustment.

Table of Contents

List of Figures	vii
List of Tables	ix
Acknowledgements	xi
Dedication	xii
Chapter 1 - Evaluating Heat Tolerance of a Complete Set of Wheat- <i>Aegilops geniculata</i>	
Chromosome Addition Lines Using Chromosome Mapping	1
Introduction.....	1
Materials and Methods	5
Results	8
Discussion	10
References.....	25
Chapter 2 - Heat Tolerance Screening of the US genome species <i>Aegilops kotschy</i> (Boiss), and <i>Aegilops peregrina</i> (k in J. Fraser) Maire & Weiller).	31
Introduction.....	31
Materials and Methods	36
Results	38
Discussion	40
References.....	52
Chapter 3 - Developing a Comprehensive Greenhouse Drought Screening System for Wheat and its Wild Relatives	58
Introduction.....	58
Materials and methods	63
Preliminary Screening.....	63
Growth tubes	65
Data collection.....	69
Irrigation system.....	70
Data collection programming	72
Moisture treatment	72
Plant measurements.....	73

Data Analysis	76
Results	76
Discussion	78
References	115
Appendix A - Advanced Drought Screening System Parts List	122
Appendix B - CR1000 (1) Programming (EC-5; MPS-6; and Tensiometers 1-32)	124
Appendix C - CR1000 (2) Programming (Tensiometers 33-54 and Automatic Irrigation System)	161

List of Figures

Figure 1-1 Chlorophyll Index (SPAD) (left) and Photochemical Efficiency of Photosystem II (Fv/Fm) (right) of all Genotypes as a Percentage of Day Zero over Temperature Treatment Period for Experiment 1 (top) and Experiment 2 (bottom).....	
Figure 1-2 Change Point Day of Heat Treatment Genotypes for Two Experiments from Multiple Regression Analysis	24
Figure 3-1 The Entire Experimental Setup of the Drought Screening System	94
Figure 3-2 (clockwise) A View of the Split Tubes, Partially Filled with Sensors; Disassembled 1:10 Scale Model of Tube Showing Cuts in PVC pipe; Drain Components in Order of Assembly.....	
Figure 3-3 The Drill Press Jig to Securely Drill Pilot Holes in One Half of the Tubes, for Sensor Placement.	96
Figure 3-4 Sensor Placement in two Types of Moisture Treatment Tubes in Advanced Drought Screening.....	
Figure 3-5 The Assembled Calibration Tube, Immediately After Saturation	98
Figure 3-6 Sectioning the Calibration Tube for Wet Mass Measurement	100
Figure 3-7 EC-5 Calibration Curve Showing Regression of Volumetric Water Content (%) on Millivolts Volts-1 Sensor Output; $R^2=0.9954$	101
Figure 3-8 The Tensimeter, Syringe, and Tygon Tubing Apparatus Used for Calibration of Pressure Transducers, Shown Connected to a Different Style of Mini-Tensiometer for Example.....	102
Figure 3-9 The two CR1000 Dataloggers (top shelf), with 10amp DC Power Supply Visible in rear, and SDMCD16AC Relays Powering Irrigation System (bottom shelf).....	103
Figure 3-10 Seven AM1632B Multiplexers in Data Acquisition Cabinet	104
Figure 3-11 DIN Rail Terminal Assembly for Terminating 54 MPS-6 Matric Potential Sensors	105
Figure 3-12 Wiring Diagram Examples for CR1000 Dataloggers	
Figure 3-13 Data Acquisition Cabinet.....	107

Figure 3-14 Deutsch 21 Pin Round Plug used for Sensors in Drought Treatment Tubes (left) and 12 pin Rectangle Plugs used for EC-5, and Tensiometers in Well-Watered Tubes, and Irrigation System (right).....

Figure 3-15 Irrigation Manifold with 12v Solenoid Valves Supplying Each Tube109

Figure 3-16 Hole Securing ¼” Irrigation Tubing in each Growth Tube 110

Figure 3-17 (clockwise) Lower Canopy of Well-watered Treatment; Leaf Stress of Lower Canopy of Drought Treatment; Comparison of Flag Leaf Stress Symptoms in Well-watered (right) versus Drought Stress (left); Wheat Flag Leaves Rolling during Drought Stress

Figure 3-18 Root System Evaluation at the Conclusion of Experiment 1. View of Tube with Intact Root System (top), Measurement of Rooting Depth (middle) and Sieve Separation of Root System and Growth Media (bottom)

Figure 3-19 Root Volume Displacement Vessel 113

Figure 3-20 Final Measurement Comparison of Chlorophyll Index, Stomatal Resistance and Dry Biomass by Moisture Treatment for Entries 18, 36, and 311 (left) in Experiment 1. Matric Potential at Three Depths at Plant Senescence for Entries 18, 36, and 311 (right).....

List of Tables

Table 1-1 Description and Details of Genotypes Used in the Heat Tolerance Study in Controlled Environments	16
Table 1-2 F-values from Analysis of Variance for Grain-fill Duration, Seeds per Spike, and Average Seed Weight for all Genotypes	17
Table 1-3 Least Square Means of Grain-Fill Duration, Seeds per Spike, and Average Seed Weight for all Genotypes, by Temperature Treatment	18
Table 1-4 Tukey-Kramer Grouping for Grain-Fill Duration of Genotype by Temperature Treatment Least Square Means ($\alpha=0.05$)	18
Table 1-5 Differences of Least Square Means for Grain-Fill Duration with Dunnett's Adjustment for Multiple Comparisons, Chinese Spring Control.....	20
Table 1-6 Whole Genome Correlations (r) Determined by SNPs for TA 2899 Growouts, Chinese Spring, and Unrelated <i>Ae. geniculata</i> Control.....	21
Table 1-7 F-values from Analysis of Variance for Grain-Fill Duration, Seeds per Spike, and Average Seed Weight for Addition Lines and Chinese Spring only.....	22
Table 2-1 A list of all Genotypes Screened in the Heat Tolerance Study, with their Species, Origin, and Total Number of Heat Treatment Observations for Experiment 1 and 2.	46
Table 2-2 Analysis of Variance F-values and degrees of freedom for Grain-Fill Duration from Experiment 1, Experiment 2, and the Heat Treatment Observations Across Experiments. ...	47
Table 2-3 Least Square Means (lsmeans) of all Heat Treatment Observations for Grain-fill Duration (GFD) in Experiment (Exp.) 1, and Paired Heat and Optimal Treatment Observations in Experiment 2.	48
Table 2-4 Heat Stress Grain-fill Duration (GFD), Temperature Treatment Contrasts, and Relative SPAD from Experiment 1, 2, and across Experiments for Superior and Control Genotypes.	49
Table 2-5 Tukey-Kramer Means Separation for least square Mean of Grain-fill Duration for Heat Observations across Experiments.....	50
Table 2-6 Analysis of Variance F-values for Chlorophyll Index (SPAD) of Experiment 2 for Days 8, 12, and 16.	51

Table 3-1 Genotypes and Their Sources Screened in the first Preliminary <i>Aegilops geniculata</i> Drought Screening Experiment	84
Table 3-2 Genotypes and their Source Screened in the Second Preliminary Drought Experiment	86
Table 3-3 Genotypes used in Advanced Drought Screening, with Source and Collection Information	87
Table 3-4 Final Nutrient Concentrations in Nutrient Solution Applied through Irrigation	88
Table 3-5 Simple Statistics for <i>Ae. geniculata</i> Entries in Drought Experiment 1 only, by Moisture Treatment	89
Table 3-6 Analysis of Variance p-value Significance Results for Select Response Variables in Experiment 1 for <i>Ae. geniculata</i> only, and Combined Analysis of all Entries for Fixed Effects of Moisture Treatment, Genotype, and their Interaction.....	90
Table 3-7 Significant Factors in a Linear Regression Model for Biomass of both Moisture Treatments, with all Entries in Experiment 1, Maximized for R ²	91
Table 3-8 Mean Physiological Responses of Genotypes in Experiment 1. Mean Osmotic Adjustment; Relative Final Stomatal Resistance, Chlorophyll Index, Biomass, and Grain- Fill Duration.....	92
Table 3-9 Mean of Select Genotypic Responses, by Moisture Treatment for Experiment 1	93

Acknowledgements

Without the love, upbringing, and support of my family, I wouldn't be where I am today. Thanks to my Mom, Dad, and Molly for everything.

My wife Megan has been supportive, helpful, understanding, loving, forgiving, and cheerful throughout this entire process. Her technical assistance and knowledge of soil science was also critical in developing the Drought Screening system. She has helped me take measurements, calibrate sensors, move plants, interpret data, and countless other tasks. Crockett and I are so much better because of her, and would otherwise be hungry, cranky, and busy most of the time.

Dr. Allan Fritz has been an outstanding mentor, and inspiring teacher. I have learned about running a successful program while maintaining what's most important in life. I will do my best to implement and pass along what you have taught me.

Shaun Winnie was integral in designing and building all aspects of the Drought Screening system. The system wouldn't be what it is without his expertise and dedication. Thanks for working long hours giving time and resources to helping make this ambition a reality.

If Dr. Gerard Kluitenberg were paid for what his advice and guidance was worth in designing the Drought Screening system, he could probably retire early. Thanks to him for agreeing to help the wheat breeders pretend to be soil scientists. If not for his generous mentoring and expertise, this system would still be a vision.

Dr. Mary Beth Kirkham has always been more than willing to help in any way possible, including references, advice, and loaning equipment. I very much appreciate her time and enthusiasm to help.

The entire wheat breeding project has been great to work and grow with. Allan, Andy, Angie, Shaun, Kim, Shyamal, Chris, Sarah, Marshall, Cristiano, Seth, Anju and all of our undergraduate help have contributed to this work in so many ways.

There has not been a safe place to hide in Throckmorton Hall from the Drought Screening system. We have gotten important help from so many people that need recognition. Allan Fritz, Shaun Winnie, Gerard Kluitenberg, Mary Beth Kirkham, Megan Green, Angie Matthews, Marshall Clinesmith, Andy Auld, Seth Filbert, Kim Suther, Eduardo Santos, Fred Caldwell, Cathie Lavis, Kyle Stropes, Decagon Harris Fellowship, Colin Campbell, Campbell Scientific, WGRC NSF, I/UCRC Student Research Grant Program, Hartnett-Nippert Lab in Biology, Environmental Manufacturing Inc. These folks have contributed greatly to the success of this project.

Lastly, thank you to the friends and plant breeding mentors who have helped along the way. My education has been a long time coming. Dr. Mark Campbell, Dr. Carl Griffey, Dr. Marla Barnett, and Dr. Greg Berger have been crucial to my education.

Dedication

This dissertation is dedicated to the memory of my grandfather, Carl E. Green. Learning and living off the land were lifelong passions of his that inspired me from an early age. From a childhood in a horse drawn buggy, to coming home from World War II to find tractor technology had reached the farm, he saw some amazing changes in his life. He would be amazed at what we can do now. He showed me what hard work looked like, but always had time to make a whistle from a willow branch or see if the fish were biting.

Chapter 1 - Evaluating Heat Tolerance of a Complete Set of Wheat- *Aegilops geniculata* Chromosome Addition Lines Using Chromosome Mapping

Introduction

Wheat (*Triticum aestivum* L.) is an important crop in the Great Plains of the United States. Approximately 15% of the hard red winter wheat produced in the U.S. is grown in Kansas and, together with Texas, Oklahoma, Nebraska, and Colorado, makes up the largest contiguous area of low rainfall winter wheat cultivated in the world (Tack et al., 2015). This geographic area experiences many temperature variations, which can affect the yield of wheat in a given cropping season. Two main contributors to yield loss are freezing temperatures in the fall and high temperatures (heat) during the spring growth period (Tack et al., 2015). Many growth stages during the life of the wheat plant are susceptible to temperature extremes, but temperature extremes surrounding anthesis and the grain-fill period are known to have a profound impact (Farooq et al., 2011; Pradhan et al., 2012a; Barkley et al., 2013; Prasad and Djanaguiraman, 2014). Optimal temperatures for grain-fill were summarized in a review by Farooq et al. (2011) who reported that the optimal temperature is $21.3^{\circ}\text{C} \pm 1.27^{\circ}\text{C}$, which represents 12 studies on the subject. Heat stress is common in Kansas and the southern Great Plains. The prevalence of extremes and temperature variability is expected to worsen in the future (Barkley et al., 2013). The Intergovernmental Panel on Climate Change suggest an average temperature increase of 0.85°C annually during the period from 1880 to 2012, with greater annual increases since 1950 (Pachauri et al., 2014). Post-anthesis heat shock occurs when temperatures exceeding 32°C

occur during the late reproductive phases and during the grain-fill period (Wardlaw and Wrigley, 1994).

Heat stress decreases the grain yield of wheat by several factors. A primary response of heat stress is early leaf senescence (Blum, 1988; Al-Khatib and Paulsen, 1990; Yang et al., 2002). Heat stress also inhibits leaf photosynthesis primarily as a result of thylakoid membrane damage (Al-Khatib and Paulsen 1984; Ristic et al., 2007) and the electron transport mechanisms in Photosystem II (Prasad et al., 2008a). The effect of heat stress is the acceleration of development and growth at all stages (Shpiler and Blum, 1986; Farooq et al., 2011). The yield component most affected by post-anthesis heat stress is kernel size, as all other yield components have been determined by this point (Yang et al., 2002). Post-anthesis heat stress decreases kernel size as a result of decreasing grain-fill duration, even though heat increases the grain filling rate (Prasad et al., 2006). The increased rate of grain fill does not usually compensate for the decrease in grain filling time (Shpiler and Blum, 1990; Prasad et al., 2008b). Heat stress may also cause or exacerbate moisture stress, as evapotranspiration increases with high temperature.

Many studies have attempted to quantify the effect of heat stress on yield loss, both experimentally and empirically, by using historical data. Stone and Nicholas (1994) reported a yield loss of up to 23% on an individual kernel level after a post-anthesis heat shock of only a few days. Streck et al. (2005) reported a grain weight loss of 1.5 mg for every 1°C increase above 20°C. As Yang et al. (2002) point out, a better estimate of heat tolerance in controlled environments when comparing genetic stocks is leaf senescence, because yield can be obscured by other genetic differences. Broader estimates of the effect of heat stress and increasing mean temperature are more common, and historical weather data is more easily utilized to make generalizations and projections based on past trends with reported grain yield. In a study

comparing wheat variety trials with historical weather data over 26 years, Barkley et al. (2013) reported a grain yield decrease of 21% for every 1°C increase in projected mean temperature. The same study reported that elevated spring temperatures had one of the most pronounced impacts on yield. In simulations reported by Tack et al. (2015), the upper threshold for the spring growth period was reported as 34°C, with each additional degree day resulting in a 7.6% decrease in grain yield.

In addition to grain yield loss, the decrease in kernel size may also decrease grain volume weight, leading to dockage of cash price paid to producers. Elevated temperatures can also have a negative impact on end-use quality of wheat (Blumenthal et al., 1993, Blumenthal et al., 1995, Stone and Nicholas, 1994) in the form of the weakening of dough properties.

Genetic improvement is the critical mechanism for coping with heat stress, because cultivar selection is one of the best ways in which a producer can adapt to heat stress. Improvement of heat tolerance per se can be difficult, because associated traits with heat tolerance are likely quantitative. Additionally, genetic progress for heat tolerance can be difficult to identify because of the base level of tolerance, which exists for wheat cultivars which have been bred in stressful environments like the Great Plains (Paulsen, 1994). It was recently suggested that newer varieties grown in the Great Plains carry less tolerance to heat stress, and that there appears to be a tradeoff between yield potential and heat tolerance (Tack et al., 2015). The two primary mechanisms by which plants cope with heat stress are tolerance and avoidance (Levitt, 1980). An important avoidance mechanism is early maturing varieties. This has been noted as important to avoiding terminal and continual stress, which is common in South Asia (Mondal et al., 2013), Australia, and globally (Bogard et al., 2014). It has long been recognized as important for evading late season heat shock and late season drought stress in the Great Plains

(Reitz and Salmon, 1959). Additional avoidance mechanisms, which may be employed are based on maximizing light interception by establishing adequate ground cover and leaf stay-green to offset the effects of decreased leaf size (Cossani and Reynolds, 2012) although these strategies are more effective for terminal stress conditions.

Before specific mechanisms can be studied and elucidated, genetic sources of heat tolerance must be identified. *Aegilops* species have been used to introduce novel sources of resistance to biotic as well as abiotic stresses. Specifically, *Aegilops geniculata* (Roth, syn *Aegilops ovata*) shows great promise for use in wheat improvement, as multiple disease resistance genes have been identified from the species (Gill et al., 1985; Kurapathy et al., 2007; Liu et al., 2011), and it has shown promise for abiotic stresses such as heat and drought tolerance (Zahaviera et al., 2001, Pradhan et al., 2012a). The species is an annual, self-pollinating, allotetraploid ($2n=4x=28$) with a genome designation $U^gU^gM^gM^g$. It represents a wide range of adaptation, with most accessions originating from the Mediterranean area. In a study of reproductive heat stress from the *Aegilops* genus, Pradhan et al. (2012a) identified two moderately tolerant accessions of *Ae. geniculata*. This included TA2899, an accession originating from Israel and held by the Wheat Genetics Resource Center at Kansas State University (WGRC). Previous and unrelated work by Friebe et al. (1999) yielded a full set of chromosome addition lines using TA2899 and Chinese Spring wheat. The availability of a full set of chromosome addition lines should facilitate the identification of the chromosome(s) possessing the reported heat tolerance by Pradhan et al. (2012a). Introgression of the trait from the addition line would be simpler because direct crosses with wheat would be possible. The objective of this study was to identify the chromosome(s) which contributed to heat tolerance in TA2899 by comparing the high temperature and optimal temperature response of the full set of

14 wheat-*Ae. geniculata* chromosome addition lines and Chinese Spring. Known heat tolerant and susceptible wheat cultivars were included as controls to quantify the levels of heat tolerance.

Materials and Methods

Chromosome addition lines were obtained from the Wheat Genetics Resource Center at Kansas State University. Fourteen chromosome addition lines (Table 1-1), *Chinese Spring*, two heat tolerant checks, *Ventnor* (Yang et al., 2002) and *Jefimija* (Ristic et al., 2007), and two heat sensitive checks, *Jagger* and *U1275* (Talukder et al., 2015) were germinated on germination paper which was wetted with a solution containing 5g liter⁻¹ terraclor (Quintozene) wettable powder fungicide. U1275 is a germplasm line developed by the USDA Hard Winter Wheat Genetics unit, which is a TAM 107 backcross derivative with the *Lr39* gene from *Aegilops tauschii* Coss. Two-to-three days after germination, the seminal roots of each seedling were removed and fixed in ice water overnight. Roots were fixed in a solution of 3 parts ethanol (99% v/v) to 1 part glacial acetic acid. After one week, roots were acetocarmine (1% carmine, 45% acetic acid) stained and the root tip caps were extracted and squashed. The addition line for chromosome 5U^g was maintained as a monosomic addition. All other addition lines were maintained as disomic addition lines. Chromosome counts were completed to identify at least 4 plants of monosomic addition line (TA7666). Roots of disomic addition lines were kept in the acetic acid-ethanol solution for future analysis. The disomic addition lines are meiotically stable with an approximately 90% transmission rate (Bernd Friebe, personal communication). Chromosome counts were not completed on disomic addition lines. After a 24 h recovery period at 4°C, the seedlings were transplanted into Sungro Professional Growing Mix (Sungro Horticulture, Agawam, MA). Plants were vernalized at 4.4°C for three weeks to ensure any vernalization requirement was met and to allow time for chromosome counts of the monosomic

addition line. Seedlings were transplanted into 15.24 cm diameter, 2.45 L round pots (Nursery Supplies Inc, Orange, CA) with two plants per pot. Plants received a 16 h photoperiod with controlled 21°C daytime temperatures and 15.5°C nighttime temperatures. Light intensity in the greenhouse from artificial lights was around $400 \mu\text{M m}^{-2} \text{ s}^{-1}$, plus ambient light. Plants were well watered to avoid any low-moisture stress. At jointing (Feekes 6) (Large, 1954), plants were tethered to bamboo stakes to avoid lodging. Pots were treated with Marathon systemic granular insecticide (1% imadicloprid, OHB) at rate of 1.4 g per pot to prevent insect damage. Plants were randomized as pairs of two pots per genotype in the greenhouse, and completely randomized in growth chambers. All measurements were based on the phenology of the primary tiller, which was tagged at spike emergence. Two pots of each genotype were grown adjacently in the greenhouse until 10 days after anthesis (Feekes 10), which was noted by anther extrusion. At that point, the pair was split, and pots were transferred to a temperature treatment chamber with one entering a high temperature chamber (35°day/30°night, 15 h photoperiod) and one entering an optimal temperature chamber (25°day/20°night, 15 h photoperiod). For experiment 1, Conviron E15 growth chambers with CMP 3244 controls were used. A square wave control with the thermoperiod matching the photoperiod was used. For experiment 2, newer chambers were acquired and Conviron PGR15 chambers with CMP 6050 controllers were used. A sinusoidal control with matching thermoperiod and photoperiod were used. For both experiments, the maximum temperature was maintained for 4 hours during the temperature treatment. Because the chamber was the experimental unit and no further true replication was possible with only two chambers, each genotype was repeated for 2 observational units, which were then averaged for a single value from each experiment for the analysis. Following a 16 day temperature treatment, pots were returned to their places in the greenhouse. Physiological readings were initiated on the

fourth day of temperature treatment, and taken every other day thereafter until tiller death, which was noted by complete flag leaf senescence or physiological maturity (yellow uppermost peduncle), whichever came first. Physiological measurements consisted of chlorophyll index, as measured by SPAD (Konica-Minolta SPAD 502 Plus; Spectrum Technologies, Aurora, IL), which measures leaf greenness and is correlated to chlorophyll content (Markwell et al., 1995) and photochemical efficiency of Photosystem II (PS II), measured by Fv/Fm variable fluorescence (Optosciences OS-30P+ handheld Fluorometer) which measures active photosystem II receptors and is correlated to photosynthetic leaf health and heat stress (Maxwell and Johnson, 2000; Ristic et al., 2007). Chlorophyll index readings were taken as an average of 3 points on the flag leaf of the main tiller (near culm, mid-sheath, near the tip), on the adaxial surface of the leaf. Fv/Fm readings were obtained with the handheld fluorometer on the adaxial surface of the same main tiller flag leaf as near to the culm as possible after a 30 minute dark adaptation. Grain-fill duration was derived as the total number of days from anthesis to tiller death. To compare the genotypic effect of heat tolerance, contrasts were calculated as the difference between least square (ls) means of the optimal minus the heat treatment. Spikelet number and seeds per spike were recorded at maturity. Seed weight per spike was obtained after 5 days of drying at 37°. Average individual seed weight was derived from seeds per spike and seed weight per spike.

SAS 9.3 (SAS Institute, 2013) was used for statistical analysis. The Glimmix procedure was used for an analysis of variance. Experiment (n=2), entry (n=19), temperature treatment (n=2), and their two-way interactions were all analyzed as fixed effects. Tukey's HSD was used for multiple comparisons. Dunnett's adjustment for multiple comparisons of means was used with the genotype Chinese Spring as the control, because it was the base genome for the addition lines in the study. A multiple regressions change point analysis of chlorophyll index and

photochemical efficiency of PS II for genotypes by experiment was completed using Proc Reg in SAS (SAS, 2016) to detect the day during physiological measurements where the slope of the response curve changed to become negative (Schwarz, 2015).

Results of the experiment called into question whether the accession tested by Pradhan et al. (2012a) was the same accession used by Friebe et al. (1999) to develop the addition lines. As a result, the seed requested from the WGRC for this study, seeds of the original spikes donated to the WGRC, as well as seed from each subsequent growout were grown for analysis. DNA extraction was performed on bulked leaf tissue from 2 plants using the BioSprint 96 DNA Plant Kit (Qiagen) with the BioSprint 96 Workstation (Qiagen). Genotyping-by-sequencing was used to identify single nucleotide polymorphisms (SNPs) in the extracted DNA following the methods of Poland et al. (2012). Markers with more than 70% missing data were discarded. The remaining SNPs were numerically coded as 1 for homozygotes of the most frequent allele, 0 for heterozygotes and -1 for homozygotes of the less frequent allele. Correlations between genotypes were compared for all available growouts. (Table 1-6).

Results

An analysis of variance of all genotypes for the three primary response variables of grain-fill duration, seeds per spike, and average seed weight is presented in Table 1-2. For grain-fill duration the factors experiment, genotype, and temperature treatment were all found to significantly differ. Analysis of variance for the same three response variables with only Chinese Spring and the addition lines is presented in Table 1-7. Excluding the wheat control genotypes was done to independently explore whether an addition line(s) significantly varied from Chinese Spring and to measure interactions between sources of variability while excluding the

inconsistent performance of wheat controls like U1275 and Ventnor (Figure 2). In this analysis of only the addition lines, temperature was the only significant source of variability.

Because grain-fill duration under heat stress is an indicator of tolerance it is of primary importance. Least square means (lsmeans) for genotypes by temperature treatment for the three primary response variables are presented in Table 1-3. The range of the chromosome addition lines in the heat treatment for grain-fill duration was from 12 to 24 days, with Chinese Spring averaging 21.25 days. Despite this apparent variability, lsmeans were compared in a pairwise Tukey-Kramer means separation, and no addition line was found to differ from Chinese Spring (Table 1-4) despite eight lines having an lsmean for grain fill duration, which was nominally higher than Chinese Spring. Only Ventnor (heat tolerant check) in the optimal temperature and the heat treatment lines TA7656, TA7664, TA7665, and U1275 were found to be different from each other. All addition lines were statistically similar to each other, and to Chinese Spring (Table 1-4, Table 1-7). In comparing the average difference between temperature treatments for each genotype (Table 1-3), there were six addition lines, which showed a small difference for grain fill duration between heat and optimal temperature, none were found to be statistically significant. A Dunnett multiple comparison test with Chinese Spring as a control group is presented in Table 1-5, which shows that no chromosome addition lines varied from Chinese Spring. For the response variable seeds per spike, genotype, experiment, and the interaction of experiment and genotype were found to differ ($p < 0.05$) (Table 1-2). For the response variable average seed weight, genotype, temperature, and the interaction of temperature and genotype were found to significantly differ ($p < 0.05$).

The significant genotype by experiment interaction for all genotypes was also detected in the change point analysis (Figure 2). The values presented as the change point were calculated by

averaging the change point day of chlorophyll index and photochemical efficiency of PS II for genotype by experiment. The wheat controls were inconsistent between experiment 1 and 2 for both measurements. The comparison between the wheat and addition lines suggests that there was no superior source of heat tolerance in the addition lines, as Jefimija consistently had a higher change point date, and Ventnor in experiment 2 was superior to the addition lines for both physiological measures. No addition line had a consistently greater change point day than Chinese Spring.

Discussion

To identify a chromosome significantly contributing to heat tolerance, two conditions must be met. First, the chromosome addition line must be significantly different from Chinese Spring. Otherwise, the alien chromatin is having no detectable effect beyond the hexaploid wheat background plus any experimental error. A significant variance between Chinese Spring and an addition line could indicate a positive or negative effect on heat tolerance. Secondly, if an addition line is found to differ from Chinese Spring, then its heat tolerance can be assessed with response variables like grain fill duration. If the mean response for an addition line is superior to Chinese Spring, then a small difference between temperature treatments for a given genotype could indicate heat tolerance. This result would suggest that the heat stress treatment did not significantly alter the optimal temperature response variables of grain fill duration, seeds per spike, and average seed weight. The represented *Aegilops geniculata* chromosome in the addition line could then be investigated as a potential source of heat tolerance.

The positive control cultivars Ventnor and Jefimija were previously reported as possessing heat tolerance (Ristic et al., 2007; Talukder et al., 2015; Narayanan et al., 2016 a and b). Because these sources of tolerance are present in hexaploid wheat, any novel sources of

tolerance from the tertiary gene pool would need to be clearly superior in order to warrant the work required for gene introgression into an adapted background. Even though the average grain fill duration of Ventnor and Jefimija were superior, they were statistically similar to most of the addition lines (Table 1-3). The heat susceptible check U1275 (Talukder et al., 2015) did fall into the lowest means group. The insignificant differences between the positive heat controls and the Chinese Spring derivatives did not necessarily indicate that the positive controls did not perform as previously reported. Alternatively, it was noted that Chinese Spring may contain higher than expected level of heat tolerance, supported by the performance of the addition lines as well as Chinese Spring having a longer grain fill duration than U1275 (Table 1-3).

For the grain fill duration analysis of variance of only addition lines, temperature was the only significant difference (Table 1-7), providing evidence of the lack of heat tolerance conferred by the *Ae. geniculata* chromatin.

Genotypes were found to significantly differ for seeds per spike and average seed weight. Despite the lack of statistically significant differences between addition lines, there appear to be slight differences in plant responses under stress. However, there are a number of morphological and physiological differences between addition lines, which could be contributing to variability in grain fill and seed weight. Genotypic differences between seed number was also expected because of the documented differences in spike type (Friebe et al., 1999). Additionally, in examining pairwise comparisons between genotypes for seeds per spike and average seed weight, no addition lines performed better than Chinese Spring for heat tolerance, as all significant variability was a negative effect in the addition line (data not shown).

Variance differences for average seeds per spike between experiments should not have been affected by differences between growth chambers. However, a significant difference was

detected (Table 1-2, Table 1-7). It is likely conditions before temperature treatment while in the greenhouse affected number of seeds per spike. The first experiment was completed in the late spring of 2013, while the second experiment was completed in late fall of the same year. Slight seasonal variability in the greenhouse conditions may well have affected the number of seeds per spike. Because of significant differences for seed number, it could be expected that a significant difference for seed weight would be found due to compensation between yield components (McNeal et al., 1978). No addition lines were found to perform better than Chinese Spring for either seeds per spike or average seed weight, despite differences (Table 1-7), indicating a negative effect in the addition lines.

In examining Dunnett's multiple comparison for grain fill duration in Table 1-4, one interesting observation was the average effect of the addition lines compared with Chinese Spring. Because the only difference between the addition lines and Chinese Spring is each single alien chromosome, the estimates in Table 1-5 can be viewed as the effect of the *Ae. geniculata* chromosome on Chinese Spring. The sum of these differences between the addition lines and Chinese Spring equate to -1.25 days in the heat treatment, indicating an overall neutral or slightly negative effect of *Ae. geniculata* chromatin on Chinese Spring. However, in the optimal treatment the sum was 11.25 days, meaning the addition lines on average were 0.8 days greater than Chinese Spring for grain fill duration. If the *Ae. geniculata* accession does not possess exceptional heat tolerance but does increase the vigor of the Chinese Spring addition lines under optimal temperatures, any small effects would be more difficult to detect because of the positive contribution to grain fill.

The physiological data for all genotypes was collected to better understand significant trends over time in heat tolerant addition lines. The photochemical efficiency of PS II and

chlorophyll index are quantitative measures of plant health, and are highly correlated to photosynthetic efficiency and heat stress responses (Ristic et al., 2007). Change point values for both measurements by experiment were analyzed. A correlation between parameters in experiment 1 was $r=0.69$, and $r=0.82$ for experiment 2. This supports the conclusion by Ristic et al. (2007) that the two measures are highly correlated measures of plant health.

Each genotypes chlorophyll index and fluorescence on treatment day 4, 8, 12 and 16 are shown as a percentage of their day 0 value in Figure 1. For the change point analysis, the presence of negative and slightly positive effects of the *Ae. geniculata* chromatin was also detected. The inconsistency of the wheat control genotypes is also easily visualized in Figure 2. There were no addition lines which had superior performance in heat stress. TA7657 showed a crossover interaction between experiments (Figure 2). This addition line has a very open floret structure, making anther extrusion a difficult indicator of anthesis. Treatment initiation was adjusted in experiment 2 to account for this factor, which may have contributed to the interaction between experiments. If differences in grain fill duration had been detected, a heat tolerant genotype might have either a high value for change point day, or a less negative slope for the period after the change point.

The lack of differences between Chinese Spring and the addition lines could be because any genetic variation for heat tolerance is quantitative and, therefore, not expressed in individual chromosomes added to the Chinese Spring background. If only one genome contains a tolerance gene, then genes which are present in TA2899 may also be having a lesser effect in the wheat genetic background because of dosage effects relating to only one homolog being present in each addition line.

Another possibility is that heat tolerance was not expressed in the addition lines is that TA2899 was not heat tolerant as reported previously (Pradhan et al., 2012a). During the screening of the entire collection of *Aegilops geniculata* for heat tolerance, the accession tested as TA2899 from the WGRC was first observed to have an abnormal spike architecture. Personal communication on *Aegilops* morphology with local experts and van Slageren (1994) suggested that the accession might have been *Aegilops peregrina*, another allotetraploid with a U^PU^PS^PS^P genome designation.

In the analysis of all available sources of TA2899, four entries (TA2899d, e, f &h) were significantly less correlated to the original TA2899 (a &b), and the seed source for the production of the addition lines (TA 2899c) in the work by Friebe et al. (1999) (Table 1-6). The four entries in question were highly related to each other, and interestingly, more highly correlated with Chinese Spring ($r=0.2$) than the original sources of TA2899 ($r=0.06$). This may also support the presence of an S genome, which is closely related to the B genome of wheat (Salse et al., 2008).

The marker data were consistent with the morphological data, which confirmed four growout sources (TA2899d, e, f &h) were different from *Ae. geniculata* based on heading date and spike morphology. Among them were the seed source for the current work on *Ae. geniculata* and the seed requested for the study by Pradhan et al. (2012a), which is TA2899d in Table 1-6. As further confirmation, genomic in situ hybridization was performed with total M and U genome DNA as probes primers to confirm that these plants were in fact not *Ae. geniculata*. The present speculation is that accession actually was *Aegilops peregrina*. The original source of TA2899 (2899a in Table 1-6) was also screened for heat tolerance using the same program

settings as the current study in a Conviron E15 growth chamber. It appeared to have very poor tolerance to heat stress (data not shown).

In conclusion, no source of heat tolerance was identified in the chromosome addition lines with TA2899. This was most likely due to identification of heat tolerance in a different genotype TA2899 by Pradhan et al. (2012a), which is not the source of *Aegilops geniculata* used to produce the chromosome addition lines by Friebe et al., (1999). This illustrates a great challenge when maintaining a germplasm collection and working with wild relatives. The tolerance source identified by Pradhan et al. (2012a) is currently being investigated to validate its potential use in wheat improvement. The method of screening chromosome addition lines remains a valid tool to quickly identify desirable alleles from wild relatives of wheat. Regardless of prior reports of heat tolerance from TA 2899, experiments such as this should be done with valuable genetic resources like chromosome addition lines. Additionally, the documented use of *Ae. geniculata* in wheat improvement (Gill et al., 1985; Kurapathy et al., 2007) and prior work suggesting superior abiotic stress tolerance (Zaharieva et al., 2001; Pradhan et al., 2012a, Pradhan et al., 2012b) supported the investigation of the addition lines. Though the conclusions of this work do not support further investigation of heat tolerance from this source, the experiments results are relevant to prevent other researchers from following the same logical path.

Table 1-1 Description and Details of Genotypes Used in the Heat Tolerance Study in Controlled Environments

Entry	Type	<i>Ae. geniculata</i> Chromosome	Entry	Type	<i>Ae. geniculata</i> Chromosome
TA 7655	DA†	1M ^g	TA 7662	DA	1U ^g
TA 7656	DA	2M ^g	TA 7663	DA	2U ^g
TA 7657	DA	3M ^g	TA 7664	DA	3U ^g
TA 7658	DA	4M ^g	TA 7665	DA	4U ^g
TA 7659	DA	5M ^g	TA 7666	MA‡	5U ^g
TA 7660	DA	6M ^g	TA 7667	DA	6U ^g
TA 7661	DA	7M ^g	TA 7688	DA	7U ^g
Ventnor	Heat Tolerant	Check	Jagger	Heat Susceptible	Check
Jefimija	Heat Tolerant	Check	U1275	Heat Susceptible	Check
Chinese	Addition	Check			
Spring	Recipient				

† Disomic Addition Line

‡ Monosomic Addition Line

Table 1-2 F-values from Analysis of Variance for Grain-fill Duration, Seeds per Spike, and Average Seed Weight for all Genotypes

Source	Degrees of Freedom	Grain Fill Duration	Seeds/Spike	Average Seed Weight
Experiment	1	5.4*	5.76*	3.3
Genotype	18	2.48*	19.17***	13.01***
Experiment * Genotype	18	0.53	3.39**	2.99**
Temperature Treatment	1	72.49***	0.08	215.26***
Genotype*Temperature Treatment	18	0.55	1.25	1.53
Experiment * Temperature Treatment	1	0.51	0.04	1.94

* p<.05

** p<.01

*** p<.001

Table 1-3 Least Square Means of Grain-Fill Duration, Seeds per Spike, and Average Seed Weight for all Genotypes, by Temperature Treatment

Genotype	Temperature Treatment†	Estimate (days)	Letter‡	Average Seed			
				Seeds per Spike		Weight	
				Optimal	Heat	Optimal	
				0°	25°/20°	35°/30°	25°/20°
Ventnor	O	40	A				
Jefimija	O	36.8	B A				
U1275	O	34.5	B A				
Jagger	O	34.5	B A				
7658	O	33	B A	3	30.3	0.0296	0.0401
7661	O	32.8	B A	3	31.5	0.0134*	0.0293*
7655	O	31.8	B A C	5	26.0	0.0187*	0.0282*
7666	O	31.5	B A C	5	26.0	0.0187*	0.0282*
7662	O	30.5	B A C	1	14.5	0.0207	0.0288
TA 7662	23.0	30.5	24.5	22.0	0.0213	0.0269	
Ventnor	23.0	40.0	21.0	21.3	0.0264	0.0380	
TA 7657	22.8	29.0	32.0	36.8	0.0204	0.0243	
TA 7659	22.3	29.0	27.5	17.8	0.0224*	0.0324*	
Jagger	22.3	34.5	32.0	28.5	0.0276	0.0371	
TA 7688	22.0	28.3	31.5	33.5	0.0186	0.0228	
TA 7655	21.8	31.8	14.3	12.0	0.0197	0.0297	
Chinese Spring	21.3	27.8	30.0	34.3	0.0231	0.0289	
TA 7667	20.8	27.0	7.3	11.3	0.0217	0.0294	
TA 7660	20.5	25.5	45.0	38.5	0.0138*	0.0259*	
TA 7663	20.3	26.5	29.5	23.5	0.0179	0.0250	
U1275	20.0	34.5	21.0	19.0	0.0255*	0.0396*	
TA 7656	19.5	27.5	19.8	23.5	0.0163	0.0215	
TA 7665	19.0	23.3	18.3	14.5	0.0226	0.0340	
TA 7664	12.0	21.5	33.3	32.5	0.0169	0.0242	

*= p<0.05 using contrast for temperature treatment

Table 1-4 Tukey-Kramer Grouping for Grain-Fill Duration of Genotype by Temperature Treatment Least Square Means (alpha=0.05)

7657	O	29	B	A	C
7659	O	29	B	A	C
7688	O	28.3	B	A	C
Chinese Spring	O	27.8	B	A	C
7656	O	27.5	B	A	C
7667	O	27	B	A	C
7663	O	26.5	B	A	C
Jefimija	H	26.5	B	A	C
7660	O	25.5	B	A	C
7666	H	24	B	A	C
7665	O	23.3	B	A	C
Ventnor	H	23	B	A	C
7662	H	23	B	A	C
7661	H	23	B	A	C
7658	H	23	B	A	C
7657	H	22.8	B	A	C
7659	H	22.3	B	A	C
Jagger	H	22.3	B	A	C
7688	H	22	B	A	C
7655	H	21.8	B	A	C
7664	O	21.5	B	A	C
Chinese Spring	H	21.3	B	A	C
7667	H	20.8	B	A	C
7660	H	20.5	B	A	C
7663	H	20.3	B	A	C
U1275	H	20	B		C
7656	H	19.5	B		C
7665	H	19	B		C
7664	H	12			C

† H= Heat Treatment, O=Optimal Treatment

‡ LS-means with the same letter are not significantly different ($\alpha=.05$)

Table 1-5 Differences of Least Square Means for Grain-Fill Duration with Dunnett's Adjustment for Multiple Comparisons, Chinese Spring Control

Heat Treatment			Optimal Treatment		
35°/30°			25°/20°		
Genotype	Difference [†]	Adj. P [‡]	Genotype	Difference	Adj. P
7655	0.50	1.00	7655	4.25	0.96
7656	-1.50	0.89	7656	0.00	1.00
7657	1.50	0.89	7657	1.25	1.00
7658	2.00	0.79	7658	5.50	0.87
7659	1.25	0.94	7659	1.50	1.00
7660	-0.50	1.00	7660	-2.00	1.00
7661	2.00	0.79	7661	5.25	0.90
7662	1.75	0.84	7662	2.75	1.00
7663	-0.75	0.99	7663	-1.00	1
7664	-9.25	0.23	7664	-6.00	0.81
7665	-2.25	0.74	7665	-4.25	0.98
7666	3.00	0.61	7666	4.00	0.99
7667	0.00	1.00	7667	-0.75	1.00
7688	1.00	0.97	7688	0.75	1.00
Jagger	1.25	0.94	Jagger	7.00	0.65
Jefimija	5.50	0.37	Jefimija	9.25	0.33
U1275	-1.00	0.97	U1275	7.00	0.65
Ventnor	2.00	0.79	Ventnor	12.50	0.09

† Taken as difference between lsmeans of each genotype minus Chinese Spring

‡ Adjusted p value

Table 1-6 Whole Genome Correlations (r) Determined by SNPs for TA 2899 Growouts, Chinese Spring, and Unrelated *Ae. geniculata* Control

Genotype	Description	TA 10437	TA 2899a	TA 2899b	TA 2899c	TA 2899d	TA 2899e	TA 2899f	TA 2899g	TA 2899h	TA 2899i	TA 2899j	TA 2899k	TA 2899l	TA 2899m	TA 2899n	TA 2899o
TA 10437	Unrelated <i>Ae. geniculata</i>	1.00															
TA 2899a	Original Donation	0.92	1.00														
TA 2899b	Original Donation	0.93	0.98	1.00													
TA 2899c	Addition Line Donor	0.91	0.97	0.98	1.00												
TA 2899d	Growout 1-2	0.69	0.68	0.68	0.67	1.00											
TA 2899e	Growout 2-1†	0.68	0.68	0.68	0.67	0.98	1.00										
TA 2899f	Growout 3-2	0.69	0.68	0.68	0.68	0.98	0.98	1.00									
TA 2899g	Growout 4-1	0.89	0.95	0.95	0.94	0.66	0.66	0.66	1.00								
TA 2899h	Growout 6-3	0.69	0.68	0.68	0.67	0.98	0.98	0.98	0.65	1.00							
TA 2899i	Growout 7-2	0.93	0.98	0.99	0.97	0.68	0.68	0.68	0.95	0.68	1.00						
TA 2899j	Growout 8-2	0.92	0.98	0.98	0.97	0.67	0.67	0.68	0.95	0.67	0.98	1.00					
TA 2899k	Growout 11-2	0.92	0.98	0.99	0.97	0.68	0.67	0.68	0.95	0.67	0.98	0.98	1.00				
TA 2899l	Growout 13-2	0.92	0.98	0.99	0.97	0.68	0.68	0.68	0.95	0.68	0.99	0.98	0.98	1.00			
TA 2899m	Growout 14-1	0.93	0.98	0.99	0.98	0.68	0.68	0.69	0.95	0.68	0.99	0.98	0.99	0.99	1.00		
TA 2899n	Growout 15-1	0.93	0.98	0.99	0.97	0.68	0.68	0.69	0.95	0.68	0.99	0.98	0.98	0.99	0.99	1.00	
TA 2899o	Growout 15-2	0.92	0.98	0.99	0.97	0.67	0.67	0.67	0.95	0.67	0.98	0.98	0.98	0.98	0.99	0.99	1.00
Chinese Spring	Chinese Spring wheat	0.08	0.06	0.06	0.06	0.20	0.20	0.20	0.06	0.20	0.06	0.06	0.06	0.06	0.06	0.06	0.06

† Seed source for study by Pradhan et al., 2012.

Table 1-7 F-values from Analysis of Variance for Grain-Fill Duration, Seeds per Spike, and Average Seed Weight for Addition Lines and Chinese Spring only

	Grain Fill Duration	Seeds/Spike	Average Seed Weight
Experiment	3.09	5.98*	0.72
Genotype	1.86	25.72***	8.81***
Experiment* Genotype	0.5	4.04**	2.28
Temperature Treatment	42.50***	0.02	281.97***
Genotype*Temperature Treatment	0.18	1.69	2.93*
Experiment * Temperature Treatment	0.06	1.46	0.02

* p<.05

** p<.01

*** p<.001

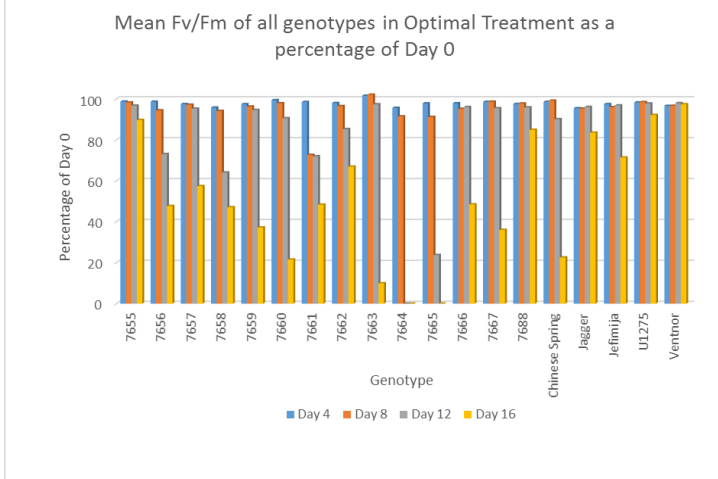
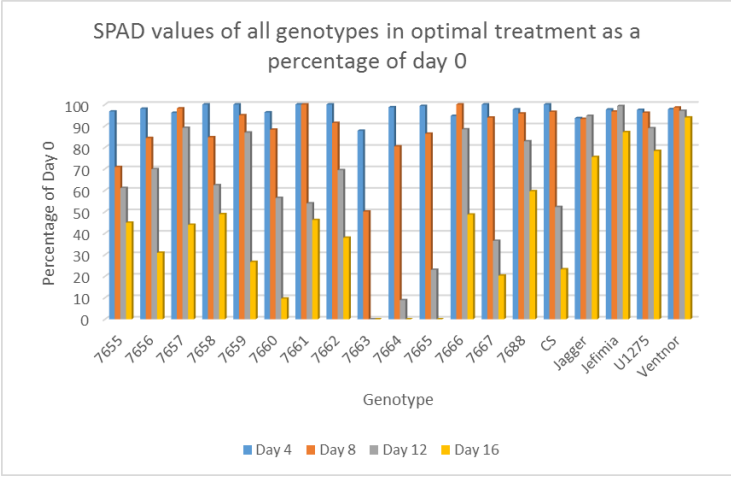
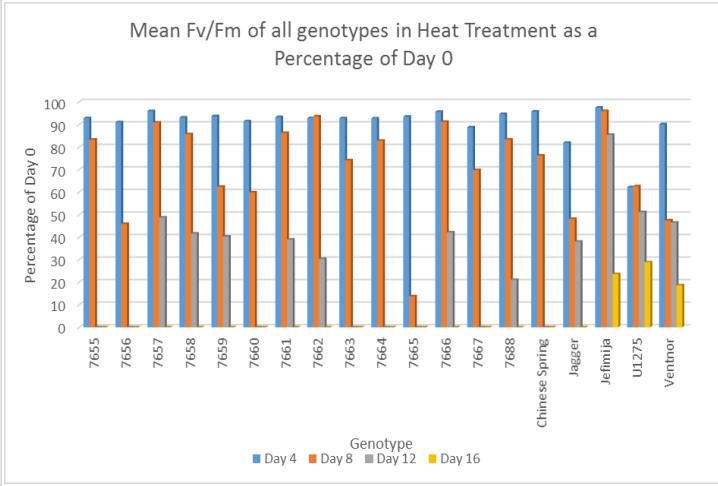
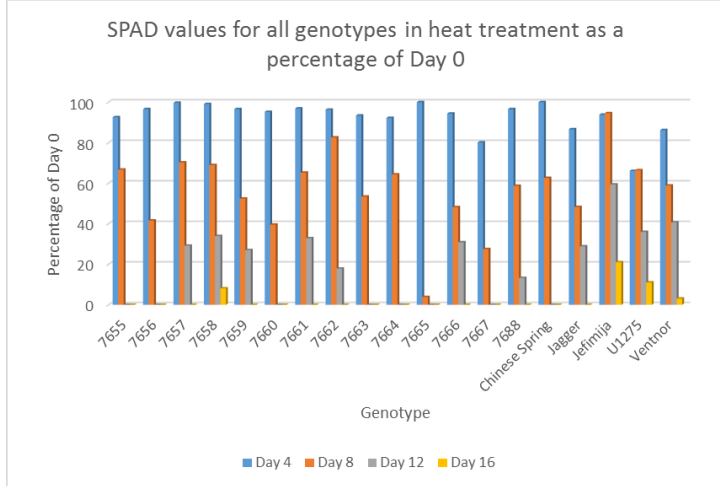


Figure 1-1 Chlorophyll Index (SPAD) (left) and Photochemical Efficiency of Photosystem II (Fv/Fm) (right) of all Genotypes as a Percentage of Day Zero over Temperature Treatment Period for Experiment 1 (top) and Experiment 2 (bottom)

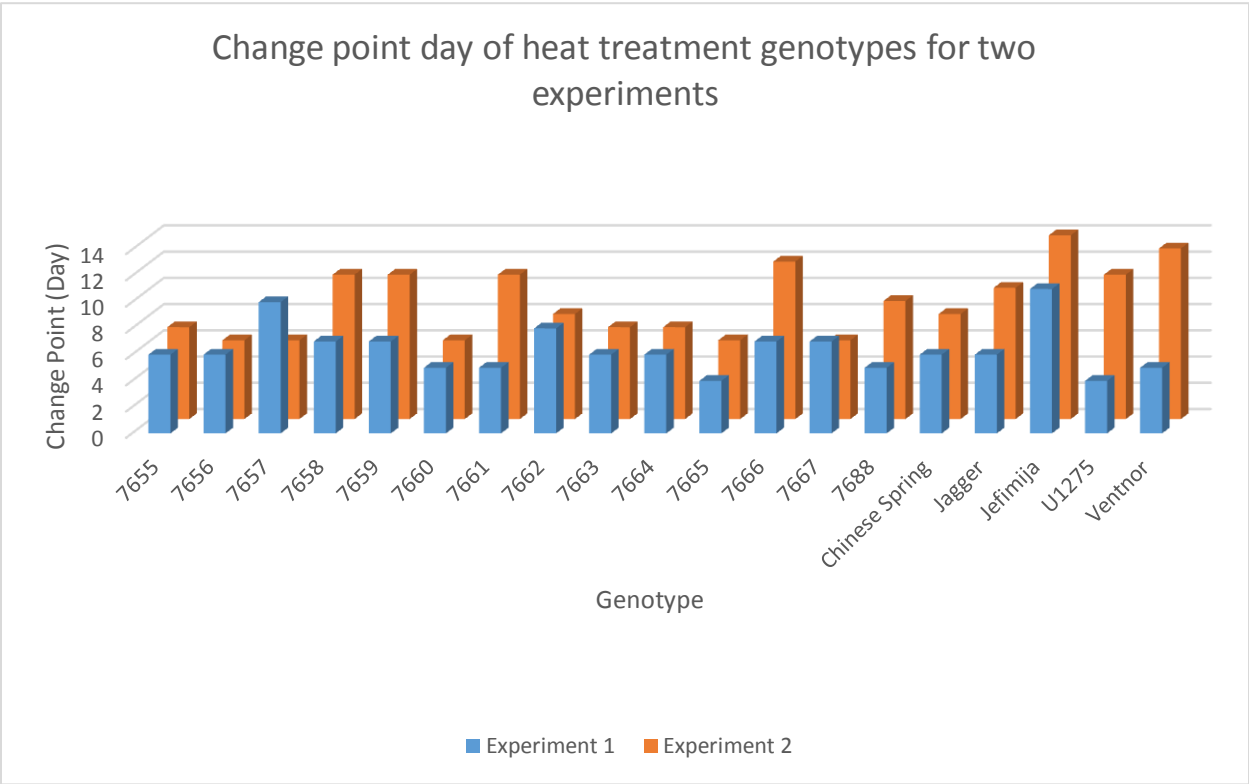


Figure 1-2 Change Point Day of Heat Treatment Genotypes for Two Experiments from Multiple Regression Analysis

References

- Al-Khatib, K., and G.M. Paulsen. 1984. Mode of High Temperature Injury to Wheat during Grain Development. *Physiologia Plantarum* 61: 363-368.
- Al-Khatib, K., and G.M. Paulsen. 1990. Photosynthesis and Productivity during High-temperature Stress of Wheat Genotypes from Major World Regions. *Crop Science* 30: 1127-1132.
- Barkley, A., J. Tack, L.L Nalley, J. Bergtold,, R. Bowden,, and A.K. Fritz. 2013. Weather, Disease, and Wheat Breeding Effects on Kansas Wheat Varietal Yields, 1985 to 2011. *Agronomy Journal* 106: 227-235.
- Blum, A., 1988. Plant breeding for stress environments. CRC Press, Inc., Boca Raton. 201 p.
- Blumenthal, C. S., E.W. R. Barlow, and C.W. Wrigley. 1993. Growth Environment and Wheat Quality: the Effect of Heat Stress on Dough Properties and Gluten Proteins. *Journal of Cereal Science* 18: 3-21.
- Blumenthal, C., F. Bekes, P.W. Gras, E.W.R. Barlow and C.W. Wrigley. 1995. Identification of Wheat Genotypes Tolerant to the Effects of Heat Stress on Grain Quality. *Cereal Chemistry* 72: 539-544
- Bogard, Matthieu, C. Ravel, E. Paux, J. Bordes, F. Balfourier, S.C. Chapman, J. Gouis, and V. Allard. 2014. Predictions of Heading Date in Bread Wheat (*Triticum aestivum* L.) Using QTL-Based Parameters of an Ecophysiological Model. *Journal of Experimental Botany* 65: 5849-5865.
- Cossani, C. M., and M.P. Reynolds. 2012. Physiological Traits for Improving Heat Tolerance in Wheat. *Plant Physiology* 160: 1710-1718.

- Farooq, M., H. Bramley, J.A. Palta, and K.H. Siddique. 2011. Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Critical Reviews in Plant Sciences* 30: 491-507.
- Friebe, B. R., N.A. Tuleen, and B.S. Gill. 1999. Development and Identification of a Complete Set of *Triticum aestivum*-*Aegilops geniculata* Chromosome Addition Lines. *Genome* 42: 374-380.
- Gill, B. S., H. C. Sharma, W. J. Raupp, L. E. Browder, J. H. Hatchett, T. L. Harvey, J. G. Moseman, and J. G. Waines. 1985. Evaluation of *Aegilops* Species for Resistance to Wheat Powdery Mildew, Wheat Leaf Rust, Hessian fly, and Greenbug. *Plant Disease* 69: 314-316.
- Kuraparthi, V., P. Chhuneja, H.S. Dhaliwal, S. Kaur, R.L. Bowden, and B.S. Gill. 2007. Characterization and Mapping of Cryptic Alien Introgression from *Aegilops geniculata* with new Leaf Rust and Stripe Rust Resistance Genes *Lr57* and *Yr40* in Wheat. *Theoretical and Applied Genetics* 114: 1379-1389.
- Large, E.C., 1954. Growth stages in cereals illustration of the Feekes scale. *Plant Pathology*. 3:128-129.
- Levitt, J. 1980. Responses of Plants to Environmental Stresses. Volume I. Chilling, Freezing, and High Temperature Stresses (No. Ed. 2). Academic Press.
- Markwell, J., J. Osterman, and J. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research* 46:467-472.
- Maxwell, K., and G.N. Johnson. 2000. Chlorophyll Fluorescence—A Practical Guide. *Journal of Experimental Botany* 51: 659-668.

- Mondal, S., R.P. Singh, J. Crossa, J. Huerta-Espino, I. Sharma, R. Chatrath, and I.K. Kalappanavar. 2013. Earliness in Wheat: A key to Adaptation under Terminal and Continual High Temperature Stress in South Asia. *Field Crops Research* 151: 19-26.
- Narayanan, S., P.J. Tamura, M.R. Roth, P.V. V. Prasad and R. Welte. 2016a. Wheat Leaf Lipids During Heat Stress: I. High Day and Night Temperatures Result in Major Lipid Alterations. *Plant, Cell and Environment*. 39:787-803.
- Narayanan, S., P.V.V. Prasad, and R. Welte. 2016b. Wheat Leaf Lipids during Heat Stress: II. Lipids Experiencing Coordinated Metabolism are Detected by Analysis of Lipid Co-occurrence. *Plant, Cell and Environment* 39: 608-617.
- Pachauri, Rajendra K., M. R. Allen, V. R. Barros, J. Broome, W. Cramer, R. Christ, J. A. Church et al. 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.*
- Paulsen, G. M. 1994. High Temperature Responses of Crop Plants. In: *Physiology and Determination of Crop Yield*. Eds. Boote, K. J., J. M. Bennett, T. R. Sinclair, and G. M. Paulsen. 365-389.
- Poland, J., J. Endelman, J. Dawson, J. Rutkoski, S. Wu, Y. Manes, et al. 2012. Genomic Selection in Wheat Breeding using Genotyping-by-Sequencing. *The Plant Genome Journal*. 3:103-113.
- Pradhan, G. P., P.V.V. Prasad, A.K. Fritz, M.B. Kirkham, and B.S. Gill. 2012a. High Temperature Tolerance in Species and its Potential Transfer to Wheat. *Crop Science* 52: 292-304.

- Pradhan, G. P., P.V.V Prasad, A.K. Fritz, M.B. Kirkham, and B.S. Gill. 2012b Response of *Aegilops* Species to Drought Stress during Reproductive Stages of Development. *Functional Plant Biology* 39: 51-59.
- Prasad, P.V.V., K.J., Boote, L.H. Allen, J.E. Sheehy, and J.M.G Thomas. 2006. Species, Ecotype and Cultivar Differences in Spikelet Fertility and Harvest Index of Rice in Response to High Temperature Stress. *Field Crops Research* 95: 398-411.
- Prasad, P. V.V., S.R. Pisipati, R.N. Mutava, and M.R. Tuinstra. 2008a. Sensitivity of Grain Sorghum to High Temperature Stress during Reproductive Development. *Crop Science*. 48: 1911-1917.
- Prasad, P.V.V., S.R. Pisipati, Z. Ristic, U. Bukovnik, and A.K. Fritz. 2008b. Impact of Nighttime Temperature on Physiology and Growth of Spring Wheat. *Crop Science* 48: 2372-2380.
- Prasad, P.V.V., and M. Djanaguiraman. 2014. Response of Floret Fertility and Individual Grain Weight of Wheat to High Temperature Stress: Sensitive Stages and Thresholds for Temperature and Duration. *Functional Plant Biology* 41: 1260-1269.
- Reitz, L. P., and S.C. Salmon. 1959. Hard Red Winter Wheat Improvement in the Plains: a 20-Year Summary (No. 1192). US Dept. of Agriculture.
- Ristic, Z., U. Bukovnik, and P.V. V. Prasad. 2007. Correlation between Heat Stability of Thylakoid Membranes and loss of Chlorophyll in Winter Wheat under Heat Stress. *Crop Science* 47: 2067-2073.
- SAS Institute, 2013. 9.3 foundation for 64-bit Microsoft Windows. SAS Inst., Cary, NC.
- SAS Institute, 2016. 9.4 foundation for 64-bit Microsoft Windows. SAS Inst., Cary, NC.
- Salse, Jérôme, V. Chagué, S. Bolot, G. Magdelenat, C.Huneau, C.Pont, H. Belcram, A. Couloux, S. Gardais, A. Evrard, and B. Segurens. 2008. New Insights into the Origin of the B

- Genome of Hexaploid Wheat: Evolutionary Relationships at the SPA Genomic Region with the S genome of the Diploid Relative *Aegilops speltoides*. *BMC Genomics*. 9: 1-12.
- Schwarz, C.J. 2015. Regression-Hockey Sticks, Broken Sticks, Piecewise, Change Points. In: Course Notes for Beginning and Intermediate Statistics. Available at <http://www.state.sfu.ca/~cschwarz/CourseNotes>. Retrieved 2 March 2016.
- Shpiler, L., and A. Blum. 1986. Differential Response of Wheat Cultivars to Hot Environments. *Euphytica* 35:483-492.
- Shpiler, L., and A. Blum. 1990. Heat Tolerance for Yield and its Components in Different Wheat Cultivars. *Euphytica* 51: 257-263.
- Stone, P. J. and M.E. Nicolas. 1994. Wheat Cultivars Vary widely in their Responses of Grain Yield and Quality to Short Periods of post-Anthesis Heat Stress. *Functional Plant Biology* 21: 887-900.
- Streck, N. A. 2005. Climate Change and Agroecosystems: the Effect of Elevated Atmospheric CO₂ and Temperature on Crop Growth, Development, and Yield. *Ciência Rural*, 35:730-740.
- Tack, Jesse, A. Barkley, and L.L Nalley. 2015. Effect of Warming Temperatures on US Wheat Yields. *Proceedings of the National Academy of Sciences* 12.22:6931-6936.
- Talukder, S. K., P.V.V. Prasad, T. Todd, M.A. Babar, J. Poland, R. Bowden, and A.K. Fritz. 2015. Effect of Cytoplasmic Diversity on post Anthesis Heat Tolerance in Wheat. *Euphytica* 204: 383-394.
- van Slageren, M. W. 1994 "Wild Wheats: a Monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen Agricultural University Papers 94-7.

Wardlaw, I. F., and C.W. Wrigley. 1994. Heat Tolerance in Temperate Cereals: An Overview. *Functional Plant Biology* 21: 695-703.

Yang J, R. R. Sears, B. Gill, and G. Paulsen. 2002. Growth and Senescence Characteristics Associated with Tolerance of Wheat-Alien Amphiploids to High Temperature under Controlled Conditions. *Euphytica* 126:185–193

Zaharieva, M., E. Gaulin, M. Havaux, E. Acevedo, and P. Monneveux. 2001. Drought and Heat Responses in the Wild Wheat Relative Roth. *Crop Science* 41: 1321-1329.

Chapter 2 - Heat Tolerance Screening of the US genome species *Aegilops kotschy* (Boiss), and *Aegilops peregrina* (k in J. Fraser) Maire & Weiller).

Introduction

Wheat (*Triticum aestivum*, L.) is cultivated worldwide, and many areas are subject to temperature extremes which limit production (Asseng et al., 2015; Tack et al., 2015). Globally, weather extremes threaten cereal production, with high temperature extremes primarily affecting grain yield (Lesk et al., 2016). In a global analysis of yields over the past 50 years, heat stress decreased yield by an average 9% in years that it occurred (Lesk et al., 2016). Average global temperature and variability is expected to continue to increase (Solomon, 2007; Barkley et al., 2013). As wheat yields continue to be affected by these trends, genetic improvement for high temperature tolerance must be addressed, particularly in the Great Plains (Barkley et al., 2013; Asseng et al., 2015; Tack et al., 2015).

A recent study from Australia reported annual change of flowering date for wheat cultivars in production of -0.074 days yr⁻¹ for the period from 1957-2010 (Zheng et al., 2016). Climate modeling data used by the authors suggest that the magnitude of change could increase in the next 30 years, and some liberal estimates suggested that the average life cycle of the crop could decrease by up to two weeks in that same time period (Zheng et al., 2016).

High temperatures during the grain fill period are known to have a profound impact on yield, with the optimal temperature being 21.3°C ±1.27°C (Farooq et al., 2012). Temperatures can exceed 34°C during grain fill in Kansas and the Great Plains, which shortens the grain fill

period (Al-Khatib and Paulsen, 1984). In addition to prematurely ending photosynthesis from early leaf senescence, starch synthesis is altered at high temperatures (Keeling et al., 1993; Jenner, 1994). These factors primarily decrease kernel weight (Yang et al., 2002). Modeling for high temperature stress during grain fill is predicted to result in profound yield losses in Europe, resulting in greater losses than drought stress (Semenov and Shewry, 2011).

Reynolds (2009) points out that, from a physiological perspective, the three primary physiological components of yield are light interception, radiation use efficiency, and partitioning of assimilates. Cossani and Reynolds (2012) examined adaptive traits under those three categories which support a plant during heat stress. Stay-green, canopy temperature depression, thermo-stability, leaf glaucousness, assimilate remobilization, and maintenance of high temperature starch synthesis are a few examples mentioned which are employed in wheat breeding (Cossani and Reynolds, 2012). Ultimately, green leaf area, functioning photosynthesis, and accumulation of biomass being assimilated into grain are what is needed of wheat plants in grain fill stress. But, interactions of these traits and over-reliance on one may compromise another. For example, as Fokar et al. (1998) point out, breeding for so-called “stay green” types may eliminate genotypes which can complete grain fill with reserves which are remobilized from the stem (Blum, 1998). Genotypes which possess this trait have a propensity for accelerated leaf senescence under stress (Blum, 1994; Fokar et al., 1998). Selection under stress for genotypes which possess adaptive mechanisms allowing them to produce biomass for grain yield is the ideal breeding approach.

Because of a number of confounding stresses found in natural environments, it may be preferable to conduct screening for high temperature stress in controlled environments such as growth chambers and greenhouses. However, few growth chamber experiments have focused on

post-anthesis heat shock in wheat (Gibson and Paulsen, 1999). As those authors point out, many growth chamber experiments to that point had utilized overly-controlled methods, such as removal of all secondary tillers, and sowing at artificially low plant populations. Since then, most growth chamber screenings have focused on anthesis stage heat stress (Ristic et al., 2007; Pradhan et al., 2012; Narayanan et al., 2016a and b) with few studies of wheat or *Aegilops* grain-fill stress (Talukder et al., 2015). Because the genetic basis of heat tolerance is poorly understood (Cossani and Reynolds, 2012), screening genotypes under stress and examining known avoidance and tolerance traits is needed. Chlorophyll content under stress, which can be estimated with SPAD (Ristic et al., 2007) is highly correlated to plant health and leaf senescence. It is an effective screening approach because yield and yield components are not particularly relevant in controlled environments, or when working with unadapted germplasm like wild relatives (Yang et al., 2002).

Breeding for superior heat tolerance is the best method for improvement, because variety selection is the only management decision on the farm level that can affect performance under heat stress. Wild relatives of wheat present opportunities for genetic improvement, and examples of alien introgression for cultivar improvement were summarized by Friebe et al. (1996), and have been increasingly successful as molecular marker tools have aided in introducing small compensating pieces of alien chromatin (Qi et al., 2007). *Aegilops* species have been used extensively for introgression of novel traits into cultivated wheat for many years (Badaeva et al., 2004). Among the species in this genus, the US genome species of *Aegilops peregrina* (Hack in J. Fraser) Maire and Weiller) and *Aegilops kotschy* (Boiss) have been investigated for various traits but there are few examples of genes which were identified in these species and made it into commercial wheat cultivars. The earliest references were studies on the dormancy controlled by

gibberellins in the hull [Wurzbürger and Koller (1976), Wurzbürger and Leshem (1969), Wurzbürger et.al (1974, 1976).] It is reported that the caryopses contain low alpha amylase activity. Each spikelet normally contains two seeds, a small and a large, with germination inhibited in the small caryopsis and normal in the larger.

Both *Ae. peregrina* and *Ae. kotschy* have been used in studies examining meiotic pairing with wheat. A report of variation in the *Ph1* gene was described by Ozkan and Feldman (2001) in crosses with *peregrina* and Chinese Spring substitution lines, among other alien backgrounds. Crosses with rye (*Secale cereale*, L.) and *Ae. kotschy* were investigated by Kwiatek et al (2012) in order to develop bridging hybrids to introduce novel traits into triticale (*x Triticosecale*). A method to induce pairing with wild relatives was described by Sheikh et al. (2016) using wheat lines monosomic for 5B was tested with both *Ae. kotschy* and *Ae. peregrina*.

A few examples of genetic improvement of end use quality using the US genome species have been documented as well. A novel high molecular weight glutenin gene from *kotschy* was described by Ma et al. (2013). Prior introgression of alien genes to increase baking quality was described by Hsam and Zeller (2001). Improvement of nutritional quality was targeted by Tiwari et al. (2010) by increasing grain iron and zinc content from *Ae. kotschy*.

US-genome species have been utilized for biotic and abiotic stress tolerance as well. In a seedling stage screening by Emon et al. (2012), tolerance to high Boron concentration was discovered in both *Ae. peregrina* and *Ae. kotschy*. Two examples of genes which have been successfully introduced into hexaploid wheat are reported by Marais et al. (2005, 2008). They report introgression of leaf rust (*Puccinia triticina* Erickss) resistance gene *Lr54* and stripe rust (*Puccinia striiformis* var *striiformis* Westend) resistance gene *Yr37* from an Israeli accession of *Ae. kotschy* to chromosome 2D of wheat (Marais et al., 2005). Another novel leaf rust resistance

gene, *Lr59*, was introduced from *Ae. peregrina* (Marais et al., 2008) with a greatly shortened segment of alien chromatin (Pirsevedi et al., 2015). One prior study aimed at abiotic stress tolerance was reported by Liu et al. (2015). They screened a number of chromosome addition lines from wild relatives of wheat and found that two *Ae. peregrina* chromosome addition lines contained the highest levels of drought tolerance in their study.

Prior heat screening of *Aegilops* species have focused on *Aegilops geniculata* (Roth) (Zaharieva et al., 2001) in addition to *Ae. longissima* (Schweinf. & Muschl.), *Ae. searsii* (Feldman & Kislev ex Hammer), *Ae. speltoides* (Tausch), and *Ae. caudata* (L.) (Pradhan et al., 2012). Heat tolerance was observed to vary widely both between and within species by both authors.

In the study by Pradhan et al. (2012), moderately high heat tolerance was reported in TA2899, which was reported in their work as an accession of *Ae. geniculata* ($U^gU^gM^gM^g$, $2n=4x=28$). It was later discovered to be a US genome species, likely *Ae. peregrina* (Chapter 1). To avoid confusing the reports in this study with the accession TA2899 which is *Ae. geniculata*, the likely *Ae. peregrina* accession is referred to as “TA2899” in this report. For this reason, and because there was no prior report of a collection of *Ae. peregrina* or *Ae. kotschyi* being screened for high temperature tolerance, these species were examined as potential sources of novel heat tolerance. Therefore, the objectives of this study were to screen the available collection of *Ae. kotschyi* and *Ae. peregrina* for post-anthesis heat tolerance in controlled environments for potential novel sources of tolerance into common wheat.

Materials and Methods

The entire available collection of the US-genome species *Ae. peregrina* and *Ae. kotschyi* were obtained from the Wheat Genetics Resource Center at Kansas State University. The accession numbers and origin, if available, are presented in Table 2-1. Wheat controls with known response to heat stress were included in the study. Two heat tolerant checks, *Ventnor* (Yang et al., 2002) and *Jefimija* (Ristic et al., 2007), and two heat sensitive checks, *Jagger* and *U1275* (Talukder et al., 2015) were used. *U1275* is a germplasm line developed by the USDA Hard Winter Wheat Genetics unit, which is a TAM 107 backcross derivative with the *Lr39* gene from *Ae. tauschii* (Coss). Seedlings were vernalized at 4.4°C for six weeks then transplanted into 9.5 cm wide, 24 cm tall, 1.65 L volume treepots (Stuewe and Sons, Corvallis, OR) with one plant per pot. The soil mix is used for all greenhouse experiments and was a combination of native soil (silty clay loam) with equal parts perlite and peat with added gypsum and carbon. Urea (46-0-0), and Osmocote Plus (15-9-12) were added to the soil mix and no further fertilization was required. Final nutrient concentrations were approximately 353 ppm NO³⁻, 146 ppm P, 490 ppm K. The pH at the time of testing was approximately 7.3. Plants received a 16 hour photoperiod with controlled 21°C daytime temperatures and 15.5°C nighttime temperatures in the greenhouse. Light intensity in the greenhouse from artificial lights was approximately 400 μM m⁻² s⁻¹, plus ambient light. Plants were well watered to avoid confounding low-moisture stress. At jointing (Feekes 6) (Large, 1954), plants were tethered to bamboo stakes to avoid lodging. Pots were treated with Marathon systemic granular insecticide (1% imadiclopid, OHB) at a rate of 1.4 g per pot to prevent insect damage. Plants were completely randomized in the greenhouse and in their respective temperature treatment growth chambers. All measurements were based on the phenology of the primary tiller, which was tagged at spike emergence. Post-anthesis

temperature treatment was initiated 10 days after anthesis, which was noted by anther extrusion. Experiment 1 was completed in fall 2014, and experiment 2 was completed in Fall 2015. For experiment 1, all entries received a high temperature stress treatment. For experiment 2, a paired treatment approach was used with half of the available pots of each genotype entering a high temperature chamber. The high temperature treatment in both experiments had a maximum daytime temperature of 35°C and a 30°C nighttime temperature with a 15 hour photoperiod. The program was modeled after a typical hot day during grain-fill in Kansas. The light and temperature were sinusoidal and gradually increased to the daily maximum, which lasted for four hours. The average daily daytime temperature in the heat treatment was 32.3°C. In experiment 2, the optimal temperature chamber had a maximum daytime temperature of 25°C, a nighttime temperature of 20°C, and average daytime temperature of 22.2°C with a 15 hr. photoperiod. If only one pot of a genotype was available, it was screened in the heat temperature treatment. Conviron PGR15 chambers with CMP 6050 controllers were used for both experiments. Because the chamber was the experimental unit and no further true replication was possible with only two chambers, each genotype was repeated for 2 to 4 observational units (Table 2-1), which were averaged from each treatment for the analysis. Following a 16 day temperature treatment, pots were returned to the greenhouse. SPAD readings were initiated on the fourth day of temperature treatment, and taken every fourth day until tiller death, which was noted by complete flag leaf senescence or physiological maturity (yellow uppermost peduncle), whichever came first. Chlorophyll index, measured by SPAD (Konica-Minolta SPAD 502 Plus), measures leaf greenness and is correlated to chlorophyll content (Markwell et al., 1995). SPAD readings were taken as an average of 3 points on the flag leaf of the main tiller (near culm, mid-sheath, near the tip), on the adaxial surface of the leaf. Grain-fill duration was derived as the total number of days

from anthesis to tiller death. To compare the genotypic effect of heat tolerance, contrasts were calculated as the difference between least square (ls) means of the optimal minus the heat treatment. SAS 9.4 (SAS Institute, 2016) was used for statistical analysis. The Glimmix procedure was used for an analysis of variance. For experiment 1, genotype (n=45) was a fixed effect. For experiment 2, genotype (n=45), temperature treatment (n=2), and the two-way interactions were all analyzed as fixed effects. Tukey's HSD was used for multiple comparisons. Dunnett's adjustment for multiple comparisons of means was used with the genotype Ventnor as the control, because it showed a consistent performance across experiments. All heat temperature observations were also analyzed across experiments, with genotype (n=50) and experiment (n=2) as fixed effects. Comparison of least square means from the heat treatment observations across experiments was used to assess stability.

Results

Analysis of variance of grain fill duration for experiment 1, 2, and the heat temperature treatment across experiments is presented in Table 2-2. In both experiments, genotypes were found to differ for grain-fill duration. As expected, temperature treatments were significantly different in experiment 2. There were no interactions found to be significant, indicating reasonable stability across temperature treatments, and experiments. Genotypes varied for grain fill duration but not for Chlorophyll Index (Table 2-6). Genotype least square (ls) means by treatment and experiment are presented in Table 2-3. Average days of grain-fill by genotype is reported, with days from spike emergence to senescence ranging from 15 to 29 days in experiment 1, and from 14 to 25.5 days in experiment 2. Because the temperature treatment began 10 days after spike emergence, these values of grain fill duration indicate that only 4 observations (TA1981, TA1984, TA2677, TA2681; Table 2-3) in experiment 1 survived the entire 16 day temperature treatment period,

while the highest average response in experiment 2 was 15.5 days of high temperature stress (TA1904), Table 2-4) Unfortunately, none of the 4 genotypes which had high average responses in experiment 1 germinated for testing in experiment 2.

Genotypes were significantly different for the analysis across experiments (Table 2-2), however, in Tukey pairwise comparisons, most genotypes were paired together (Table 2-5). In evaluating performance across experiments, there were several accessions that had a nominal value greater than the wheat checks, and were reasonably stable. If greater replication or a true susceptible check were used, greater separation between genotypes may have been observed.

Top genotypes were selected based on performance across experiments, small differences in pairwise comparisons between temperature treatments in experiment 2, performance relative to the wheat controls, small differences in Chlorophyll Index across temperature treatments, and high relative SPAD values. These top genotypes are presented in Table 2-4. Contrasts between temperature treatments were analyzed for genotypes with high average days to senescence for grain fill duration and Chlorophyll Index. Genotypes were not found to differ overall for day 12 and 16 SPAD measurements, but contrasts between temperature treatments for genotype by temperature treatment revealed the few genotypes which did differ ($p < 0.05$), and can be seen in Table 2-4. A small or nonexistent variance between plant responses would indicate possible heat tolerance. Day 12 and 16 values for heat and optimal treatment of SPAD were averaged by genotype and the values in the heat treatment are presented in Table 2-4 as a relative percentage of the average SPAD from the corresponding optimal treatment of that genotype. For example, a genotype which has a relative SPAD value of 100% would have identical SPAD readings in both temperature treatments, but a relative SPAD of 50% would indicate an average heat treatment observation half that of the optimal temperature treatment.

A Dunnett multiple comparisons means adjustment was calculated from experiment 2 using Ventnor as the control genotype. Ventnor was previously reported to be heat tolerant, and had a more stable performance across genotypes than Jefimija (Table 2-3). Six genotypes differed ($p < 0.05$) from Ventnor in experiment 1, but all were more negative than the Ventnor control (data not shown). No genotypes varied significantly from Ventnor in experiment 2, indicating that heat tolerance from *Ae. kotschy* and *Ae. peregrina* was either equal to or less than Ventnor. High standard errors prevented significant adjusted p values in experiment 2, even with genotypes that had higher days to senescence than Ventnor (e.g. TA1901, TA1904).

Discussion

Biomass data or kernel weight data would have been informative to help separate genotypes under stress. However, significant germination and seedling vigor problems were experienced during both experiments. This led to unbalanced data between experiments as well as variability in mature plant size, which would have confounded analysis of biomass. Variability in *Ae. kotschy* could be explained by the early work of Wurzburger et al. (1969, 1976). They discuss the dormancy issues of one half of *Ae. kotschy* seeds per spikelet. For future work with this species, separation of the large and small caryopses during hand-threshing may avoid this complication. These challenges highlight the difficulty of working with wild relatives. In controlled environments, there are few procedures that can identify heat tolerance with agronomically meaningful measurements, and leaf health is the most useful when working with wild species (Yang et al., 2002). Because these species have very narrow leaf blades, variable fluorescence data collection is rarely possible, and SPAD readings can be difficult to obtain as well.

Variation in the wheat controls, both heat tolerant and heat susceptible, also made detection of heat tolerance difficult. The heat susceptible genotype U1275 (Talukder et al., 2015) had a fairly long grain fill duration in both experiments (Table 2-3) and was statistically similar to the heat tolerant checks Ventnor and Jefimija (Table 2-5). Additionally, Jefimija had a substantially poorer performance in the second experiment. Jagger was fairly consistent between experiments for grain-fill duration (Table 2-4). A lack of a true susceptible genotype makes mean separation between wild accessions more difficult, and may be because germplasm originating from the Great Plains must contain some heat tolerance (Paulsen, 1994). In evaluating these species, it appears that there are some entries which are equal to the level of tolerance that is currently in common wheat (Table 2-4). Four accessions of *Ae. peregrina* from Israel showed a higher average relative SPAD percentage than all of the wheat checks, and had grain fill duration values across experiments, exceeding the heat tolerant checks (Table 2-4), even though they were grouped similarly in a means separation (Table 2-5). TA2275 had statistically similar grain fill duration between the heat and optimal treatment in experiment 2, and had similar SPAD values under heat stress at Day 12 and Day 16. It was also very consistent between experiment 1 and 2 for grain fill, lasting 21 and 21.7 days respectively. It was noted for its robust nature, high tiller count, and large leaf area (personal observation). TA1904 had dissimilar grain fill duration between heat and optimal treatment in experiment 2, which was likely caused by its high optimal treatment grain-fill of 36 days (Table 2-3). Its 12 day relative SPAD percentage was 53.3%, and its 16 day relative SPAD percentage was still 28.9%. It had the highest consistent average grain fill in heat between experiment 1 and 2 of 24.3 days 25.5 days, respectively. It was also noted for its leaf architecture and size, robust tiller production, and spike size (personal observation). In experiment 2, it lasted 2.5 days longer than Ventnor, which

was still statistically insignificant because of a high standard error (data not shown). TA1904 was the only genotype which was statistically superior to any other accessions (Table 2-5).

TA2275, TA1904, TA1901, and TA1889 were noted for their apparent visual heat tolerance, which was noted by a slow rather than abrupt leaf senescence. Interestingly, both TA2275, and TA1901 are *Ae. peregrina* accessions from Israel, and that these accessions consistently performed better than the *Ae. kotschyi* accessions that also originated from Israel (Table 2-1, 2-3). TA1904 has an unknown origin, but came from the collection of renowned wheat geneticist Ernie Sears at the University of Missouri (personal communication with Jon Raupp, Wheat Genetics Resource Center).

There were additional genotypes which showed apparent stability between temperature treatments, and across experiments. TA1896, and “TA2899” had stable grain-fill durations across both experiments, while TA1986 and TA1903 actually had higher grain-fill durations in the heat treatment of experiment 2. These could be worthy of further examination for stable heat tolerance.

Because of extreme phenotypic variability in accessions of wild relatives, visual observations can be important for identifying genotypes which do not appear to possess negative traits that would add to linkage drag in gene introgression with wheat. TA10551 had a high mean grain fill duration across experiments, and had similar ($p < 0.05$) grain fill duration across temperature treatments in experiment 2. By examining data alone, it would appear as the top gene introgression candidate among *Ae. kotschyi* accessions. However, personal observations recorded during both experiments showed that this accession has an unusually small spike (<20 mm) which was largely infertile, possibly prolonging plant life under stress due to a very low

photosynthetic sink. In terms of agronomic improvement, it appears to offer very little compared with many *Ae. peregrina* accessions, which have very robust plant types and spikes.

This work followed Pradhan et al. (2012), which suggested that TA2899 was moderately tolerant to heat stress, as determined by a heat susceptibility index experiment performed across several *Aegilops* species. Several accessions had higher mean grain fill durations and relative SPAD values (Table 2-4), indicating that superior sources of tolerance are present in these tested U-S genome species.

Further replicated work targeting the physiological mechanism of heat tolerance may help reveal whether they differ from Ventnor and Jefimija, and if they would be agronomically desirable. Without a relative grain production measurement between putative heat tolerant genotypes, the possibility remains that photosynthetic products are not being used for accumulation of grain yield. Because these species evolved in the wild without selection for grain production, their mechanism for survival may be exclusive of grain production.

There are many potential errors that can occur when screening wild relatives for heat stress. False positives can occur by errors in early tagging of tillers at anthesis, resulting in early imposition of stress treatment. Errors in assessing leaf senescence or tiller death can also affect grain-fill estimates. Without sound physiological measurements, estimation of leaf senescence alone may overestimate the ability of the plant to photosynthesize despite apparently green tissue.

There are also several potential sources of false negatives in similar studies. Reliance on leaf senescence may prohibit detection of genotypes which assimilate grain from stem carbohydrates (Blum et al., 1994). Leaf senescence can approximate plant death, but

translocation of carbohydrates from stem tissues could also contribute to grain yield. This can be difficult to quantify unless yield is measured after defoliation (Blum et al., 1998).

The temperature used in this study was modeled after similar studies by Pradhan et al. (2012) and Ristic et al. (2007). The high nighttime temperature may make separation of genotypes difficult, as the heat stress may be severe. In plastic pots in a growth chamber, the roots of entries in a heat treatment may also be artificially heated, resulting in a greater accumulation of stress (Heckathorn et al., 2013). A small variance between temperature treatments is used as an indication of heat tolerance, so a potential false negative may result from a genotype which has an exceptionally high grain fill in the optimal temperature treatment. If a genotype does not have a genetically limited grain fill period, it may express an opportunistic grain fill period which is extended due to favorable conditions in the optimal temperature treatment. The ability to perform under both stressful and favorable conditions would be preferred for genetic improvement, so care must be taken to avoid this type of false negative. It is for this reason that emphasis in the current study was placed on plant-type observations as well as high days to senescence under heat stress. Ideally, a high relative SPAD value would indicate that the heat treatment observation is performing at similar levels as its optimal treatment counterpart, with less reliance on the overall grain fill period.

Based on a review of the literature, these species have been underutilized for abiotic stress tolerance in cultivated wheat. Increased replicates and an additional experiment with paired temperature treatment data may help to increase statistical power to detect differences between accessions. Wheat-alien amphiploids of the consistently performing *Ae. peregrina* accessions should be made to investigate potential heat tolerance and other abiotic stress transfer. Because of their genomic diversity, it is possible that wheat could be also be improved for other

traits by recombination between them. The evidence is not compelling to believe that there is a clearly superior source of heat tolerance present in these species, but detection of a significantly different tolerance appears to have been made difficult by unbalanced data sets from dormancy and germination problems, high standard error, and lack of available physiological data on many accessions. The lack of a true susceptible genotype in the current study also makes paring down the collection for replicated study a difficult task as well. These species largely originate in the Middle East (Table 2-1) in areas which regularly experience high temperature stress (Bita and Gerats, 2013; Ortiz et al., 2008). Their close relation to the B genome of wheat (Salse et al., 2008) may help facilitate genetic improvement which is agronomically competitive.

Table 2-1 A list of all Genotypes Screened in the Heat Tolerance Study, with their Species, Origin, and Total Number of Heat Treatment Observations for Experiment 1 and 2.

Genotype	Origin	species	Heat Obs.		Genotype	Origin	species	Heat Obs.	
			Exp. 1	Exp. 2				Exp. 1	Exp. 2
TA1885	Israel	<i>Ae. peregrina</i>	1	3	TA1982	Egypt	<i>Ae. kotschyi</i>	1	3
TA1886	Syria	<i>Ae. peregrina</i>	2	3	TA1983	Egypt	<i>Ae. kotschyi</i>	2	1
TA1887	Israel	<i>Ae. peregrina</i>	2	1	TA1984	Egypt	<i>Ae. kotschyi</i>	1	0
TA1888	Israel	<i>Ae. peregrina</i>	1	0	TA1985	Israel	<i>Ae. kotschyi</i>	1	1
TA1889	Israel	<i>Ae. peregrina</i>	1	2	TA1986	Israel	<i>Ae. kotschyi</i>	2	3
TA1890	Israel	<i>Ae. peregrina</i>	2	3	TA2173	Israel	<i>Ae. peregrina</i>	2	1
TA1891	Israel	<i>Ae. peregrina</i>	0	3	TA2205	Israel	<i>Ae. kotschyi</i>	1	2
TA1892	Canada	<i>Ae. peregrina</i>	2	1	TA2206	Azerbaijan	<i>Ae. kotschyi</i>	1	1
TA1893	Turkey	<i>Ae. peregrina</i>	2	2	TA2207	Uzbekistan	<i>Ae. kotschyi</i>	2	1
TA1894	Jordan	<i>Ae. peregrina</i>	2	1	TA2274	Jordan	<i>Ae. peregrina</i>	2	2
TA1895	Jordan	<i>Ae. peregrina</i>	3	3	TA2275	Israel	<i>Ae. peregrina</i>	1	3
TA1896	U.K.	<i>Ae. peregrina</i>	1	2	TA2665	Jordan	<i>Ae. kotschyi</i>	1	3
TA1897	Turkey	<i>Ae. peregrina</i>	2	1	TA2667	Jordan	<i>Ae. kotschyi</i>	2	0
TA1898	Lebanon	<i>Ae. peregrina</i>	2	2	TA2681	Jordan	<i>Ae. peregrina</i>	1	0
TA1901	Israel	<i>Ae. peregrina</i>	1	2	TA2682	Syria	<i>Ae. peregrina</i>	2	1
TA1902	Palestine	<i>Ae. peregrina</i>	1	3	TA2698	Israel	<i>Ae. peregrina</i>	1	2
TA1903	Palestine	<i>Ae. peregrina</i>	2	1	TA10550	Israel	<i>Ae. kotschyi</i>	0	1
TA1904	Unknown	<i>Ae. peregrina</i>	3	2	TA10551	Israel	<i>Ae. kotschyi</i>	2	2
TA1918	Turkey	<i>Ae. peregrina</i>	2	3	TA10855	Cyprus	<i>Ae. peregrina</i>	1	3
TA1919	Israel	<i>Ae. peregrina</i>	2	2	“TA2899”	Unknown	<i>Ae. peregrina</i>	2	3
TA1920	Turkey	<i>Ae. peregrina</i>	0	2	Jagger	U.S.A.	<i>T. aestivum</i>	2	4
TA1974	Canada	<i>Ae. kotschyi</i>	1	2	Jefimija	Serbia	<i>T. aestivum</i>	2	2
TA1978	Israel	<i>Ae. kotschyi</i>	0	1	U1275	U.S.A.	<i>T. aestivum</i>	2	3
TA1980	Israel	<i>Ae. kotschyi</i>	2	1	Ventnor	Australia	<i>T. aestivum</i>	3	2
TA1981	Egypt	<i>Ae. kotschyi</i>	1	0					

Table 2-2 Analysis of Variance F-values and degrees of freedom for Grain-Fill Duration from Experiment 1, Experiment 2, and the Heat Treatment Observations Across Experiments.

Experiment	Effect	Numerator		
		Degrees of Freedom (DF)	Den DF	Grain Fill Duration
1	Genotype	44	32	2.71**
2	Treatment	1	79	142.76***
	Genotype	44	79	1.65*
	Treatment*Genotype	35	79	1.51
Heat Across Experiments	Experiment	1	78	11.05**
	Genotype	39	78	2.02**
	Genotype*Experiment	39	78	1.28

* p< 0.05; ** p<0.01, *** p<0.001

Table 2-3 Least Square Means (lsmeans) of all Heat Treatment Observations for Grain-fill Duration (GFD) in Experiment (Exp.) 1, and Paired Heat and Optimal Treatment Observations in Experiment 2.

Genotype	species	Exp. 1	Experiment 2		Genotype	species	Exp. 1	Experiment 2	
		GFD	GFD H	GFD O			GFD	GFD H	GFD O
TA10551	<i>Ae. kotschyi</i>	25.0	20.5	27.7	TA1980	<i>Ae. kotschyi</i>	18.0	19.0	.
TA10855	<i>Ae. peregrina</i>	24.0	19.0	22.0	TA1981	<i>Ae. kotschyi</i>	18.0	.	.
TA1885	<i>Ae. peregrina</i>	16.0	22.7	29.5	TA1982	<i>Ae. kotschyi</i>	18.0	18.7	22.0
TA1886	<i>Ae. peregrina</i>	19.5	15.7	27.0	TA1983	<i>Ae. kotschyi</i>	16.5	14.0	29.0
TA1887	<i>Ae. peregrina</i>	17.5	18.0	24.5	TA1984	<i>Ae. kotschyi</i>	17.0	.	.
TA1888	<i>Ae. peregrina</i>	15.0	.	.	TA1985	<i>Ae. kotschyi</i>	17.0	19.0	41.0
TA1889	<i>Ae. peregrina</i>	26.0	22.5	28.5	TA1986	<i>Ae. kotschyi</i>	21.0	22.3	20.5
TA1890	<i>Ae. peregrina</i>	21.5	20.0	30.7	TA2173	<i>Ae. peregrina</i>	22.5	19.0	36.0
TA1892	<i>Ae. peregrina</i>	24.5	22.0	.	TA2205	<i>Ae. kotschyi</i>	29.0	16.0	34.0
TA1893	<i>Ae. peregrina</i>	23.0	21.0	28.0	TA2206	<i>Ae. kotschyi</i>	16.0	15.0	.
TA1894	<i>Ae. peregrina</i>	17.0	20.0	.	TA2207	<i>Ae. kotschyi</i>	16.0	18.0	.
TA1895	<i>Ae. peregrina</i>	22.7	19.0	33.0	TA2274	<i>Ae. peregrina</i>	22.5	17.5	26.0
TA1896	<i>Ae. peregrina</i>	22.0	22.0	23.0	TA2275	<i>Ae. peregrina</i>	21.0	21.7	27.5
TA1897	<i>Ae. peregrina</i>	25.0	14.0	26.0	TA2665	<i>Ae. kotschyi</i>	24.0	17.0	27.7
TA1898	<i>Ae. peregrina</i>	24.0	18.0	27.0	TA2667	<i>Ae. kotschyi</i>	17.0	.	.
TA1901	<i>Ae. peregrina</i>	19.0	23.5	29.0	TA2681	<i>Ae. peregrina</i>	17.0	.	.
TA1902	<i>Ae. peregrina</i>	25.0	22.7	31.0	TA2682	<i>Ae. peregrina</i>	18.5	19.0	.
TA1903	<i>Ae. peregrina</i>	17.0	18.0	14.0	TA2698	<i>Ae. peregrina</i>	22.0	21.5	30.0
TA1904	<i>Ae. peregrina</i>	24.3	25.5	36.0	“TA2899”	-	21.0	20.0	22.0
TA1918	<i>Ae. peregrina</i>	18.0	19.3	.	Jagger	<i>T. aestivum</i>	19.0	19.0	33.0
TA1919	<i>Ae. peregrina</i>	22.5	18.0	25.0	Jefimija	<i>T. aestivum</i>	25.0	18.5	24.5
TA1974	<i>Ae. kotschyi</i>	19.0	18.5	30.5	U1275	<i>T. aestivum</i>	22.0	20.3	27.0
TA1974	<i>Ae. kotschyi</i>	19.0	18.5	30.5	Ventnor	<i>T. aestivum</i>	25.7	23.0	40.0
CV							18.4	16.5	22.3

Table 2-4 Heat Stress Grain-fill Duration (GFD), Temperature Treatment Contrasts, and Relative SPAD from Experiment 1, 2, and across Experiments for Superior and Control Genotypes.

Entry	Origin	Species	GFD 1	GFD 2	Combined GFD	Exp. 2	12d	12d	16d	16d
						Temp Contrast	SPAD Pairwise	Relative SPAD	SPAD Pairwise	Relative SPAD
TA1904	Unknown	<i>Ae. peregrina</i>	24.3	25.5	24.9	≠	=	53.3%	≠	28.9%
TA1889	Israel	<i>Ae. peregrina</i>	26.0	22.5	24.3	=	=	36.0%	=	0
TA1902	Palestine	<i>Ae. peregrina</i>	25	22.7	23.8	≠	≠	9.1%	≠	0
TA10551	Israel	<i>Ae. kotschyi</i>	25	20.5	22.8	=	≠	11.9%	=	0
TA1893	Turkey	<i>Ae. peregrina</i>	23.0	21.0	22.0	=	≠	0.0%	=	0
TA1896	U.K.	<i>Ae. kotschyi</i>	22	22.0	22.0	=	=	0.0%	=	0
TA2698	Israel	<i>Ae. peregrina</i>	22.0	21.5	21.8	=	≠	0.0%	≠	0
TA2275	Israel	<i>Ae. peregrina</i>	21.0	21.7	21.3	=	=	47.2%	=	0
TA1901	Israel	<i>Ae. peregrina</i>	19.0	23.5	21.3	=	=	61.0%	≠	0
Ventnor	Australia	<i>T. aestivum</i>	25.7	23.0	24.3	≠	≠	41.4%	≠	0
Jefimija	Serbia	<i>T. aestivum</i>	25.0	18.5	21.8	=	≠	0.0%	≠	0
U1275	U.S.A.	<i>T. aestivum</i>	22.0	20.3	21.2	=	≠	8.6%	=	0
“TA2899”	Unknown	Unknown	21.0	20.0	20.5	=	=	20.5%	=	0
Jagger	U.S.A.	<i>T. aestivum</i>	19.0	19.0	19.0	≠	≠	20.3%	≠	0

≠ Statistically dissimilar in contrast or Tukey pairwise comparison for genotype by temperature treatments

= Statistically similar in contrast or Tukey pairwise comparison for genotype by temperature treatments

Table 2-5 Tukey-Kramer Means Separation for least square Mean of Grain-fill Duration for Heat Observations across Experiments

Genotype	Days	Letter [†]	Genotype	Days	Letter
1904	24.9	A	2899	20.5	BA
Ventnor	24.3	BA	2665	20.5	BA
1889	24.3	BA	1919	20.3	BA
1902	23.8	BA	2274	20.0	BA
1892	23.3	BA	1897	19.5	BA
10551	22.8	BA	1885	19.3	BA
2205	22.5	BA	Jagger	19.0	BA
1893	22.0	BA	1974	18.8	BA
1896	22.0	BA	2682	18.8	BA
Jefimija	21.8	BA	1918	18.7	BA
2698	21.8	BA	1980	18.5	BA
1986	21.7	BA	1894	18.5	BA
10855	21.5	BA	1982	18.3	BA
2275	21.3	BA	1985	18.0	BA
1901	21.3	BA	1887	17.8	BA
U1275	21.2	BA	1886	17.6	BA
1898	21.0	BA	1903	17.5	BA
1895	20.8	BA	2207	17.0	BA
1890	20.8	BA	2206	15.5	B
2173	20.8	BA	1983	15.3	B

[†] Genotypes with same letter are not statistically different ($p>0.05$)

Table 2-6 Analysis of Variance F-values for Chlorophyll Index (SPAD) of Experiment 2 for Days 8, 12, and 16.

	8d	<i>Pr>F</i>	12d	<i>Pr>F</i>	16d	<i>Pr>F</i>
	SPAD		SPAD		SPAD	
Treatment	78.13	<.0001	108.03	<.0001	99.1	<.0001
Genotype	1.02	0.4644	1.29	0.1584	1.23	0.2122
Treatment*Genotype	0.93	0.5893	1.06	0.4107	1.38	0.1224

References

- Al-Khatib, K., and G.M. Paulsen. 1984. Mode of High Temperature Injury to Wheat During Development. *Physiology. Plantarum* 61: 363-368.
- Asseng, S., F. Ewert,, P. Martre, R.P. Rötter,, D.B. Lobell, D. Cammarano, B.A. Kimball, M.J. Ottman, G.W. Wall, J.W. White, and M.P. Reynolds. 2015. Rising Temperatures Reduce Global Wheat Production. *Nature Climate Change* 5:143-147.
- Badaeva, E.D., A.V. Amosova, T.E. Samatadze, S.A. Zoshchuk, N.G. Shostak, N.N. Chikida, A.V. Zelenin, W.J. Raupp, B. Friebe, and B.S. Gill. 2004. Genome Differentiation in *Aegilops*. 4. Evolution of the U-Genome Cluster. *Plant Systematics and Evolution* 246:45-76.
- Barkley, A., J. Tack, L.L. Nalley, J. Bergtold,, R. Bowden, and A.K. Fritz. 2013. Weather, Disease, and Wheat Breeding Effects on Kansas Wheat Varietal Yields, 1985 to 2011. *Agronomy Journal* 106:227-235.
- Bitá, C.E., and T. Gerats. 2013. Plant Tolerance to High Temperature in a Changing Environment: Scientific Fundamentals and Production of Heat Stress-Tolerant Crops. *Frontiers in Plant Science* 4:1-18.
- Blum, A., B. Sinmena, J. Mayer, G. Golan, and L. Shpiler, 1994. Stem Reserve Mobilization Supports Wheat Grain Filling under Heat Stress. *Australian Journal of Plant Physiology* 21: 771–781.
- Blum, A. 1998. Improving Wheat Grain Filling under Stress by Stem Reserve Mobilisation. *Euphytica* 100: 77-83.
- Cossani, C. M., and M.P. Reynolds. 2012. Physiological Traits for Improving Heat Tolerance in Wheat. *Plant Physiology* 160:1710-1718.

- Emon, R. M., K. Gustafson, P. J. Bebeli, M. Jahiruddin, M. S. Haque, K. Ross, and J. P. Gustafson. 2012. Screening *Aegilops-Triticum* species for Boron tolerance. *African Journal Agricultural Research* 7: 1931-1936.
- Farooq, M., H. Bramley, J.A. Palta, and K.H. Siddique. 2011. Heat Stress in Wheat during Reproductive and Grain-filling Phases. *Critical Reviews in Plant Sciences* 30:491-507.
- Fokar, M., H.T. Nguyen, and A. Blum. 1998. Heat Tolerance in Spring Wheat. I. Estimating Cellular Thermotolerance and its Heritability. *Euphytica* 104: 1-8.
- Fokar, M., A. Blum, and H.T. Nguyen. 1998. Heat Tolerance in Spring Wheat. II. Grain Filling. *Euphytica* 104:9-15.
- Friebe, B., J. Jiang, W.J. Raupp, R.A. McIntosh and B.S. Gill. 1996. Characterization of Wheat Alien Translocations Conferring Resistance to Diseases and Pests: Current Status. *Euphytica* 91: 59-87.
- Gibson, L.R. and G.M. Paulsen. 1999. Yield Components of Wheat Grown under High Temperature Stress during Reproductive Growth. *Crop Science* 39:1841-1846.
- Heckathorn, S.A., A. Giri, S. Mishra, and D. Bista. 2013. Heat Stress and Roots. 109-136. In *Climate Change and Plant Abiotic Stress Tolerance* (eds N. Tuteja and S. S. Gill), Wiley VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- Hsam, S.L.K., R. Kieffer, F.J. Zeller, 2001. Significance of *Aegilops tauschii* Glutenin Genes on Breadmaking Properties of Wheat. *Cereal Chemistry* 78:521-525.
- Jenner, C.F. 1994. Starch Synthesis in the Kernel of Wheat under High Temperature Conditions. *Australian Journal of Plant Physiology*. 21:791-806.

- Keeling, P. L., P. J. Bacon, and D. C. Holt. 1993. Elevated Temperature Reduces Starch Deposition in Wheat Endosperm by Reducing the Activity of Soluble Starch Synthase. *Planta* 191: 342-348.
- Kwiatek, M., L. Błaszczyk, H. Wiśniewska and B. Apolinarska. 2012. *Aegilops-Secale* Amphiploids: Chromosome Categorisation, Pollen Viability and Identification of Fungal Disease Resistance Genes. *Journal of Applied Genetics* 53:37-40.
- Large, E.C., 1954. Growth Stages in Cereals Illustration of the Feekes Scale. *Plant Pathology*. 3:128-129.
- Lesk, C., P. Rowhani, and N. Ramankutty. 2016. Influence of Extreme Weather Disasters on Global Crop Production. *Nature* 529:84-87.
- Liu, C., Z. Yang, and Y.G.Hu. 2015. Drought Resistance of Wheat Alien Chromosome Addition Lines Evaluated by Membership Function Value Based on Multiple Traits and Drought Resistance Index of Grain Yield. *Field Crops Research*, 179:103-112.
- Ma, C., Y. Yang, X. Li, P. Ge, G. Guo, S. Subburaj, F.J. Zeller, S.K. Hsam, and Y. Yan. 2013. Molecular Cloning and Characterization of Six Novel HMW-GS Genes from *Aegilops speltoides* and *Aegilops kotschyi*. *Plant Breeding* 132:284-289.
- Marais, G. F., B. McCallum, J.E. Snyman, Z.A. Pretorius, and A.S. Marais. 2005. Leaf Rust and Rust Resistance Genes *Lr54* and *Yr37* Transferred to Wheat from *Aegilops kotschyi*. *Plant Breeding* 124:538-541.
- Marais, G. F., B. McCallum, and A.S. Marais. 2008. Wheat Leaf Rust Resistance Gene *Lr59* Derived from *Aegilops peregrina*. *Plant Breeding* 127:340-345.
- Markwell, J., J.C. Osterman, and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 Leaf Chlorophyll Meter. *Photosynthesis Research*.46:467-472.

- Narayanan, S., P.J. Tamura, M.R. Roth, P.V. Prasad and R. Welti. 2016. Wheat Leaf Lipids during Heat Stress: I. High Day and Night Temperatures Result in Major Lipid Alterations. *Plant, Cell & Environment* 39:787-803.
- Ortiz R., H.J. Braun, J. Crossa, J.H. Crouch, G. Davenport, J. Dixon, et al. 2008. Wheat Genetic Resources Enhancement by the International Maize and Wheat Improvement Center (CIMMYT). *Genet. Resour. Crop Evolution*. 55:1095–1140
- Ozkan, H. and M. Feldman. 2001. Genotypic Variation in Tetraploid Wheat Affecting Homoeologous Pairing in Hybrids with *Aegilops peregrina*. *Genome* 44:1000-1006.
- Paulsen, G. M. 1994. High Temperature Responses of Crop Plants. In: *Physiology and Determination of Crop Yield*, 365-389. K.J. Boote, Ken J., J. Bennett, T.R. Sinclair, and G.M. Paulsen, eds. American Society of Agronomy.
- Pirseyedi, S.M., M. Somo, R.S. Poudel, X. Cai,, B. McCallum, B. Saville, T. Fetch, S. Chao, and F. Marais. 2015. Characterization of Recombinants of the *Aegilops peregrina* Derived *Lr59* Translocation of Common Wheat. *Theoretical and Applied Genetics* 128:2403-2414.
- Pradhan, G. P., P.V.V. Prasad, A.K. Fritz, M.B. Kirkham, and B.S. Gill. 2012. High Temperature Tolerance in Species and its Potential Transfer to Wheat. *Crop Science* 52:292-304.
- Qi, L., B. Friebe, P. Zhang and B.S. Gill. 2007. Homoeologous Recombination, Chromosome Engineering and Crop Improvement. *Chromosome Research* 15:3-19.
- Reynolds MP, Y. Manes, A. Izanloo and P. Langridge. 2009. Phenotyping Approaches for Physiological Breeding and Gene Discovery in Wheat. *Annals of Applied Biology* 155: 309–320.

- Ristic, Z., U. Bukovnik, U., and P.V.V. Prasad. 2007. Correlation between Heat Stability of Thylakoid Membranes and loss of Chlorophyll in Winter Wheat under Heat Stress. *Crop Science* 47:2067-2073.
- Salse, J.V. Chagué, S. Bolot, G. Magdelenat, C.Huneau, C.Pont, H. Belcram, A. Couloux, S. Gardais, A. Evrard, and B. Segurens. 2008. New Insights into the Origin of the B Genome of Hexaploid Wheat: Evolutionary Relationships at the SPA Genomic Region with the S Genome of the Diploid Relative *Aegilops speltoides*. *BMC genomics* 9: 1-12.
- SAS Institute, 2016. 9.4 foundation for 64-bit Microsoft Windows. SAS Inst., Cary, NC.
- Semenov, M.A., and Peter R. Shewry. 2011. Modelling Predicts that Heat Stress, not Drought, will Increase Vulnerability of Wheat in Europe. *Scientific Reports*, 1:66.
- Sheikh, I., P. Sharma., S.K. Verma, S. Kumar, S. Malik, P. Mathpal, U. Kumar, D. Singh, S. Kumar, V. Chugh., and H.S. Dhaliwal. 2016. Characterization of Interspecific Hybrids of *Triticum aestivum x Aegilops* sp. without 5B chromosome for Induced Homoeologous Pairing. *Journal of Plant Biochemistry and Biotechnology* 25:117-120.
- Solomon, S. 2007. IPCC 2007. Climate Change The Physical Science Basis. In AGU Fall Meeting Abstracts 1:1.
- Tack, J., A. Barkley, and L.L Nalley. 2015. Effect of Warming Temperatures on US Wheat Yields. *Proceedings of the National Academy of Sciences* 112: 6931-6936.
- Talukder, S. K., P.V.V. Prasad, T. Todd, M.A. Babar, J. Poland, R. Bowden, and A.K. Fritz. 2015. Effect of Cytoplasmic Diversity on Post Anthesis Heat Tolerance in Wheat. *Euphytica* 204:383-394.

- Tiwari, V.K., N. Rawat, K. Neelam, S. Kumar, G.S. Randhawa, and H.S. Dhaliwal. 2010. Substitutions of 2S and 7U Chromosomes of *Aegilops kotschy* in Wheat Enhance Grain Iron and Zinc Concentration. *Theoretical and Applied Genetics* 121:259-269.
- Wurzburger, J., and D. Koller. 1976. Differential Effects of the Parental Photothermal Environment on Development of Dormancy in Caryopses of *Aegilops kotschy*. *Journal of Experimental Botany* 27: 43-48.
- Wurzburger, J., Y. Leshem, and D. Koller. 1974. The Role of Gibberellin and the Hulls in the Control of Germination in *Aegilops kotschy* Caryopses. *Canadian Journal of Botany* 52: 1597-1601.
- Wurzburger, J., and Y. Leshem. 1969. Physiological Action of the Germination Inhibitor in the Husk of *Aegilops kotschy* Boiss. *New Phytologist*. 68:337-341.
- Wurzburger, J., Y. Leshem, and D. Koller. 1976. Correlative Aspects of Imposition of Dormancy in Caryopses of *Aegilops kotschy*. *Plant Physiology*, 57:670-671.
- Yang, J. R. R. Sears, B.S. Gill, and G.M. Paulsen. 2002. Growth and Senescence Characteristics Associated with Tolerance of Wheat-alien Amphiploids to High Temperature under Controlled Conditions. *Euphytica* 126:185–193
- Zaharieva, M., E. Gaulin, M. Havaux, E. Acevedo, and P. Monneveux. 2001. Drought and Heat Responses in the Wild Wheat Relative *Aegilops geniculata*, *Roth*. *Crop Science* 41:1321-1329.
- Zheng, B., K. Chenu, and S.C. Chapman. 2016. Velocity of Temperature and Flowering Time in Wheat—Assisting Breeders to keep pace with Climate Change. *Global Change Biology* 22:921-933.

Chapter 3 - Developing a Comprehensive Greenhouse Drought Screening System for Wheat and its Wild Relatives

Introduction

Drought tolerance is defined by Turner (1979) as the ability of a plant to survive moisture stress and reproduce satisfactorily. Crop plants should also maintain yield to be considered drought tolerant. While droughts may vary in severity, severe droughts are often beyond the scale of agronomic solutions (Blum, 2011). The requirement of harvestable yield proposed by Turner (1979) in the context of advanced production systems excludes catastrophic droughts which Blum (2011) better describes as political and economic problems. Yield is often limited by drought stress in the southern Great Plains of the United States of America (Musick et. al, 1994). In a recent modeling study by Tack et al. (2014), they reported that drought scenarios had the historical effect of a 22% yield reduction, while warming temperatures had an average 11% reduction. The reproductive and grain-fill periods are the most limiting periods for drought in wheat (Pradhan et al., 2012).

The effects of drought stress are numerous, but the primary effect on grain yield is a decrease in photosynthesis. This occurs due to an increase in leaf senescence (Yang et al., 2003), which is primarily triggered by stress induced leaf chlorosis (Yang 2001; Gregersen and Holm, 2007). The effect of leaf senescence on the flag leaf has a large effect on yield production, because the flag leaf is responsible for 30-50% of yield assimilation (Sylvester-Bradley et al., 1990). Accelerated leaf senescence results in a reduction of the grain fill duration (Plaut, 2004). Photosynthesis is also largely inhibited by a tolerance mechanism in response to stress. Stomatal closure, in an attempt to decrease evaporative water loss, results in a decrease in transpiration and thus decreased photosynthesis (Cornic, 2000). This survival mechanism helps plants survive

periods of severe stress, but may be mutually exclusive of improvement for genetic yield potential because it results in decreased transpiration, and thus, grain-fill.

Plants cope with drought stress in a variety of ways. Escape is a strategy often used for drought tolerance, especially in areas where terminal stress late in the growing season is a problem (Blum, 1988; Blum et al., 1989). Wheat plants may also rely on reserve carbohydrates from other plant organs, such as the stem or awns, to be translocated to grain production when faced with stress (Blum et al., 1983). An increase in Abscisic acid (ABA) hormone signaling often occurs in drought stress, and has been shown to affect many plant responses. It has been shown to promote root growth and water extraction (Chimenti et al., 2006). ABA also acts as an “early warning signal” (Blum, 2011) and is released in response to drying around upper roots, signaling leaf retardation and stomatal closure. Blum (2011) points out that these effects may be conflicting with sustained photosynthesis under stress that is required to protect yield. Therefore, in crop improvement, high ABA sensitivity should be approached with caution. Because it can restrict water losses through evaporation and transpiration, some level of ABA signaling is still likely beneficial but over-sensitivity may result in premature shutdown of photosynthesis.

Water use efficiency is a metric which is often used by agronomists to describe the biomass accumulated as a proportion of evapotranspiration. It has been described as a selection tool by some (Fischer, 1981; Condon et al., 2004). A pitfall that Blum (2009) points out, is that WUE is merely a ratio, and that it should not be used to evaluate breeding materials for cultivar development because high WUE doesn't necessarily lead to increased yield. Alternatively, he argues that genotypes which are continually selected for low transpiration will be negatively associated with yield improvement, because high, but efficient transpiration is needed for grain production. As long as no genetic improvement in photosynthetic efficiency is expected,

selection for lower yielding materials would result from consistent WUE selection. This is because lower or conservative biomass can result in a high water use efficiency, which is why he advocates the term effective use of water (EUW) coupled with biomass selection.

Osmotic adjustment is the process by which plants accumulate solutes in leaf tissue at low leaf water potentials to maintain turgor and cellular hydration (Blum, 2011). Higher levels of osmotic adjustment has been associated with deeper root growth as well (Morgan and Condon, 1986). Osmotic adjustment takes time to develop, and therefore is not an effective avoidance strategy in quick drought situations. It has been estimated that it may take at least 14 days in wheat, but less than 28 days required by most rice cultivars (Babu et al., 1999).

Genotypes having deep root systems to access water at depths are desired for drought tolerance. Rooting depth can increase drought tolerance (Xue et al., 2003; Wasson et al., 2012). Screening for root depth is difficult, because there are no reliable in situ screening methods available in the field (Farooq et al., 2012) despite there being important genotypic variation for this trait (Richards, 2006). It has been postulated that if a genotype possesses the ability to mine for water at progressive depths as stress develops, that it may not need to be capable of osmotic adjustment (Blum et al., 1999). In controlled environments, root analysis is sometimes possible. Rooting architecture is often studied with computer software capable of calculating the root length, area, and volume. This analysis can be very time consuming, and a simple estimation of rooting volume has shown to be highly correlated to the software analysis, at a much lower cost (Pang et al., 2011).

Selecting for drought tolerant genotypes in a breeding program is difficult because of the low heritability often caused by genotype by environment interaction (Farooq et al., 2012). Genotypes must be screened in drought environments, but genetic yield potential must also be

identified in less stressful environments. For this reason, effective physiological screening should accompany yield testing in breeding programs to identify potential drought tolerant genotypes.

Several approaches have been suggested to identify physiological traits highly correlated to drought tolerance. Low canopy temperature is associated with yield in drought stress (Blum et al., 1989; Pradhan et al., 2014). Leaf water potential (Kirkham et al., 1969; Kirkham, 1983; O'Toole and Cruz, 1980; Pask et al., 2012) and relative water content (Barrs and Weatherley, 1962) are often monitored under drought stress in controlled environment studies, in order to evaluate stress in a quantitative and repeatable manner (Kirkham, 2005). Drought dependent Harvest Index (Richards et al., 2002) and whole plant biomass under stress (Xue et al., 2014) are two methods for evaluating plant production under stress. Stem dry weight mass at harvest was recently suggested as a method for screening for genotypes that may translocate carbohydrate stem reserves for grain-fill (Xue et al., 2014). Field testing of grain yield with leaf desiccant such as magnesium chlorate is also used to simulate grain-filling from stem reserves (Blum, 2009).

In experimental situations, osmotic adjustment may be estimated between well-watered plants that never receive stress and drought stressed plants (Blum, 1989). It has been emphasized that the osmotic potential of the genotypes involved must be measured at similar relative water contents (Babu et al., 1989; Zhang et al., 1999; Blum, 2009). A high correlation with two previously proposed methods (Morgan's regression and Ludlow's full turgor adjustment) of assessing osmotic adjustment was shown by Zhang et al. (1999). Their rehydration method with two treatments of plants was very similar to more time-consuming methods, and consumed less plant matter (Zhang et al., 1999). Their rehydration method is also preferred for post-anthesis drought stress because it involves rehydrating the excised leaves, rather than re-watering of the experimental plants. Rehydration of leaf matter allows evaluation of leaves at similar water

content, provides enough sap to be able to read on a vapor pressure osmometer, and was experimentally shown to avoid dilution of solute content after a less than 12 hour rehydration (Babu et al., 1989).

Wild relatives of wheat have been suggested as a source of abiotic stress tolerance, in addition to disease resistance genes (Friebe et al., 1996). *Aegilops geniculata* (Roth) has been suggested as a source of drought tolerance from the tertiary gene pool of wheat (Monneveux et al., 2000; Zaharieva et al., 2001; Pradhan et al., 2012). The species has a broad range of adaptation, and has a center of origin near the cool deserts near the Mediterranean Sea. This and other *Aegilops* species are best studied in controlled environments because of adaptation, lodging, and spike shattering at maturity.

The objective of this study was to develop a screening system for wheat and its wild relatives in a controlled greenhouse environment. Because of the number of confounding stresses which may affect plant performance, drought stress must be completely isolated to identify genotypes for further investigation. The mechanism of tolerance can be simultaneously investigated by screening for physiological mechanisms of drought such as rooting traits, stomatal resistance, and osmotic adjustment. An advanced screening system with the ability to monitor above and below ground conditions could be used to investigate potential sources of drought tolerance from *Aegilops geniculata*. For genetic wheat improvement, the level of tolerance should be compared with the response of known wheat genotypes.

Materials and methods

Preliminary Screening

In spring 2014, a preliminary screening was performed on 145 entries (Table 1) from the Wheat Genetics Resource Center (WGRC) and USDA-ARS National Small Grains Collection (Aberdeen, ID) in two replications. Water was completely withheld 10 days post head emergence, and days to senescence was measured. Eighty eight *Ae. geniculata* accessions were selected based on phenotype and grain fill duration from screening 1 and screened with two wheat checks (TAM 111 and TAM 112) with three replicates in a second screening in fall 2014. Both putative tolerant and a few susceptible genotypes were retained, based on phenotype and grain fill duration. Days to senescence, biomass, and visual observations on phenotype were recorded for the second preliminary screening. For both preliminary experiments, seedlings were vernalized at approximately 4.4°C for six weeks then transplanted into 6-inch diameter, 0.65-gallon round pots (Nursery Supplies Inc, Orange, CA) with two plants per pot. Each pot contained the same amount of soil at a uniform moisture content. Plants received a 16 h photoperiod with 21°C daytime temperatures and 15.5°C nighttime temperatures. Ambient light was supplemented with artificial light in the greenhouse. Artificial light intensity was about 400 $\mu\text{M m}^{-2} \text{ s}^{-1}$. At jointing (Feekes 6), plants were tethered to bamboo stakes to avoid lodging. Pots were treated with Marathon systemic granular insecticide (1% imadicloprid, OHB) at a rate of 1.4 g per pot to prevent insect damage. A randomized complete block experimental design was used in the greenhouse with bench as the blocking factor.

Linear regression (data not shown) was used to select genotypes with high biomass and grain fill duration for the advanced drought screening. Five genotypes with diverse phenotypes, high biomass, and high grain fill duration were screened in the advanced screening with three

wheat controls (Table 3). A putative susceptible genotype of *Ae geniculata* (TA 10021) was included based on its previous poor performance. TAM 111 is a high yielding cultivar adapted to the High Plains with a record of high yield potential in moderate drought conditions, as well as high input environments (Battenfield et al., 2013). TAM 112 is a High Plains wheat cultivar which appears to be drought tolerant under more severe drought conditions, with a slightly lower yield potential in optimal environments (Pradhan et al., 2014). Santa Fe was grown on limited acreage and developed a reputation for being susceptible to drought (Watson, 2015).

Advanced screening experimental design

Seeds were planted in Profile Greens Grade (Profile Products, Buffalo Grove, IL) and kept in a warm greenhouse for three weeks before vernalization. Seedlings were vernalized at approximately 4.4°C for six weeks. They were watered at least once per week in vernalization, and fertilized after three weeks with the same nutrient solution used for the experiment.

Following vernalization, the seedlings were transplanted into growth tubes containing Profile Greens Grade growth media (Figure 1). Plants received a 12 h photoperiod for the first four weeks after transplanting, increasing to 14 h after two weeks, and 16 h six weeks after transplanting. Plants received 21°C daytime temperatures and 15.5°C nighttime temperatures. Ambient greenhouse light was supplemented with growth lights. The light intensity in the greenhouse from artificial lights (Sunblaze T5 4'x8 with Spectralux 6500K lamps) was around 775 $\mu\text{M m}^{-2} \text{ s}^{-1}$.

The advanced screening was a split block treatment design, with moisture treatment (well-watered, drought) as the main block factor and genotype as the sub-block factor. Genotypes were replicated twice in each moisture treatment. Each drought treatment tube was paired with a well-watered treatment tube based on heading date, for the purposes of

physiological comparisons across treatments. Each tube had five plants. Drought stress was imposed 10 days after anthesis, marked by spike emergence of 60% of the plants in a tube. Senescence was noted by 60% of plants with a flag leaf being no longer photosynthetically active and when a SPAD or stomatal resistance measurement was no longer possible.

Growth tubes

The experimental unit for the advanced screening was a 60-inch long section of Schedule 40 polyvinylchloride (PVC) pipe (6-inch o.d.). Each pipe was cut twice longitudinally for all but 3-inches on two sides, resulting in two halves for insertion into the coupler base (Figure 2). A third cut, perpendicular to the first two, was made at the base to free one half of the pipe, resulting in a 4-foot, 9-inch-long half which could be removed at the conclusion of the experiment for in situ root analysis. The other half of the pipe had the intact 6-inch o.d. base which was 3-inches tall. The kerf of the two longitudinal cuts was filled with 3/8-inch-thick closed-cell foam weather stripping (WJ Dennis, Elgin, IL) to seal the tube. The kerf of the perpendicular cut was filled with 1/2-inch-thick weather stripping. . Before tubes were filled with growth media, they were closed with three 6-7" hose clamps, spaced evenly along the length of the tube.

The base of the tube, which was uncut longitudinally, was placed inside a 6" PVC coupling which had a 6" round landscaping drain grate placed inside of it (Figure 2). A piece of size 60 stainless steel mesh was placed on top of the grate to retain the growth media yet still allow for free drainage. Size 60 mesh has openings which are 0.01 mm smaller than the smallest particle size of the growth media. Two arch-shaped holes were cut in the base of the PVC coupler drain using 1-1/2-inch hole saw in a drill press to allow drainage to escape the base. The

holes were drilled by clamping two couplers together end to end, and drilling one hole with the pilot bit of the hole saw inserted between the two couplers.

Holes were pre-drilled with a 3/64" drill bit and tapped with a 3/8" NPT tap at the sensor positions along the tubes (Figure 3). A 3/8" cable gland (Mencom Corporation) with strain relief was placed in each tapped hole. Sensors were oriented with the narrow edge in the "up" position, to minimize resistance to water flow, as seen in Figure 2.

Profile Greens Grade (Profile Products LLC, Buffalo Grove, IL) is a baked porous ceramic aggregate made of calcined ilite clay (Adams et al., 2014). It is used as a field amendment on golf courses, and has also been used extensively as an experimental growth media. It was studied by Steinberg et al. (2005) as a potential plant growth media for use on the international space station. Macropores drain at relatively high levels of VWC (Steinberg et al., 2005), making it well suited for a drought screening experiment. Even when packed to its maximum bulk density (0.68 g cm^{-3}), this media has an unusually high porosity and drains well. It also has a very low hydraulic conductivity at relatively high volumetric water content levels, lending it well to drought experiments. It drains rapidly, making management of water and nutrient delivery very forgiving of accidental over watering. Due to the essentially non-existent cation exchange capacity of the media, the experiment must be treated as a hydroponic experiment, with nutrient solution being delivered during each irrigation.

Growth media was packed into the tubes using four "lifts", each 15-inches in height and packed to a target bulk density of $\rho_b = 0.68 \text{ g cm}^{-3}$. The elevation for the top of each of these lifts was marked on the inner surface of the tubes. The mass M of media required for each lift was determined with the expression $M = \rho_b V(1 + \theta_g)$, where V is the volume of media in each lift and θ_g is the gravimetric water content of the media. Samples collected immediately prior to packing

were used to determine that the air-dry media had a water content of $\theta_g = 0.02 \text{ g g}^{-1}$. After adding the mass M of media for a given lift, the exterior surface of the growth tube was tapped repeatedly until the media settled to the target elevation for that lift. Packing the tubes in four lifts helped ensure uniformity in bulk density with depth, but the primary control on uniformity resulted from the fact that the target bulk density was set equal to the maximum bulk density (i.e., 0.68 g cm^{-3}) for this media (Steinberg et al., 2005).

Osmocote Plus 3-4 (15-9-12) (Scotts, Marysville, OH) was included in the top 14-inches of the tube at a 7.1 g dm^{-3} rate to supplement plant nutrient needs. Tubes were completely saturated with water immediately after being filled with the growth media.

Sensors

Three different sensors were used to monitor soil water levels during the experiment. The EC-5 (Decagon Devices, Pullman, WA) is a low-cost, rugged volumetric water content sensor. It has the smallest volume of influence of similar analog moisture content sensors from Decagon Devices, making it well suited for placement inside of the tubes without interference from the walls. The EC-5 sensors were spaced 12-inches apart at four depths inside each growth tube, beginning 12-inches from the growth media surface near the top of the tube (Figure 4). For the drought treatment tubes ($n=18$), MPS-6 matric potential sensors (Decagon Devices, Pullman, WA) were placed 3-inches below the EC-5 sensors at the top three depths (Figure 4). These are SDI-12 sensors which measure soil temperature and matric potential (Ψ_m). They have a published effective range of $-10 \text{ MPa} < \Psi_m < -9 \text{ kPa}$. To maintain adequate contact with the surrounding growth media, the MPS-6 sensors were dipped in a 200-mesh silica flour (Soil Moisture Equipment, Santa Barbara, CA) slurry at the recommendation of the sensor manufacturer. The slurry was made by mixing silica flour with water until it was viscous enough

to adhere to the ceramic disc of the MPS-6 sensor. For the well-watered tubes, a corresponding Ψ_m measurement was made at the same position in the tubes (Figure 4) with a mini column tensiometer (Soil Measurement Systems, Huntington Beach, CA). The tensiometers were custom ordered with a 16-cm-long barrel. Measurements are made automatically with a 26PC pressure transducer (Honeywell), which is included from SMS. The pressure transducer has an effective range to 5 psi, making it well suited for measurements in the well-watered treatment.

Calibration of the EC-5 sensors was necessary for the growth media used in this experiment. A calibration column was designed and constructed specifically for this task. Twenty pieces of 6-inch o.d. PVC pipe were cut to a length of 3-inches. A cable gland was installed in the center of 10 of these sections, using the procedure described above. The sections were assembled as shown in Figure 5 and held together with general purpose duct tape (Duck Brand). Each section was packed to a target bulk density of 0.68 g cm^{-3} using the same procedure employed for packing the growth tubes. An EC-5 reading was taken with the air dry Profile, having a known gravimetric water content, immediately after packing. The column was watered until water freely drained from the bottom, ensuring that it was nearly saturated. An EC-5 reading in millivolts was taken immediately after saturation. The column was then allowed to drain naturally for several days in the greenhouse under ambient conditions. Readings from the EC-5 sensors were recorded every three hours during the drainage event. Once differentiation in sensor readings was observed, the column was sectioned (Figure 6), and wet mass of the media was determined for each of the 10 sections containing an EC-5 sensor. The media from these sections was oven dried at 105°C for 72 hours and then weighed to determine dry mass. The gravimetric water content values calculated with the wet- and dry-mass data were converted to volumetric water contents by using the expression $\theta_v = \theta_g \rho_b / \rho_w$, where θ_v is the volumetric

water content ($\text{cm}^3 \text{cm}^{-3}$) and ρ_w is the density of water, taken to be 1.0 g cm^{-3} . EC-5 readings in mv/V were regressed onto volumetric water content values (expressed on a percentage basis, i.e., $\theta_v \times 100$) to obtain a calibration curve for the growth media (Figure 7).

Calibration of the tensiometer pressure transducers was checked with a tensiometer (Soil Measurement Systems, Huntington Beach, CA) to verify accuracy. A range of five suction values were drawn with a 10cc syringe and 1/8" tygon tubing connected to the tensiometer (Figure 8). Tensiometer values and pressure transducer readings were compared with linear regression to obtain an r^2 of 0.998 (data not shown), confirming that the pressure transducers had similar measurements for the range of matric potential expected in the experiment

Data collection

Data were collected with two CR1000 dataloggers (Figure 9; Campbell Scientific, Logan, UT). To configure the total number of sensors, seven AM16-32B multiplexers (Figure 10; Campbell Scientific, Logan, UT) were used. The multiplexers were run in "4x16" mode, allowing a total of 48 EC-5 sensors, or 16 tensiometers per multiplexer. Because the MPS-6 sensors are digitally addressable SDI-12, they were all wired with a 6" DIN rail (Figure 11; Campbell Scientific, Logan, UT). Wiring diagrams for the EC-5 and tensiometer multiplexers are shown in Figure 12. The system received 120 volt power from the greenhouse where it is housed, and a PS150 (Campbell Scientific, Logan, UT) 12 volt power supply was used to power the dataloggers.

A cabinet was designed and specially constructed to house the data acquisition system (Figure 13). The enclosure was sealed from dust and moisture, and included a 118 cfm, 12v exhaust fan (Jameco Electronics, Belmont, CA) to prevent humidity damage to electronic components. To allow tubes and the data acquisition system to be physically separated during

root characterization and extraction and in between experiments, Deutsch connectors (LADD, Kettering, OH) were installed on sensor wires (Figure 14). These were inserted into permanently mounted plugs on the sides of the data acquisition cabinet. The plugs and hardware are waterproof and maintain excellent connections. This allows the multiplexers and dataloggers to remain fully connected when not in use, preventing error and damage to equipment by disconnecting and reconnecting wires. A full set of parts used to install plugs on sensors is found on the parts list (Appendix 1).

Irrigation system

An automatic irrigation scheduling system utilizing the soil moisture sensors was designed to increase repeatability and consistency of the experimental protocol. The system uses the datalogger programming to assess water content from selected sensor readings at a specified time, and then initiate irrigation when the water content reading falls below a specified value. This decreases the need to manually monitor and apply water, which may need to be done several times a day during periods of rapid water uptake.

Each tube was controlled by a 1/4" 12v solenoid valve (Electronic Solenoid Valves, Holbrook, NY). The valves were controlled by three SDMCD16AC relays (Figure 9; Campbell Scientific, Logan, UT). Each channel on the SDMCD16AC relay has a toggle switch allowing the program settings to be turned off or manually overridden. The relays were controlled by one CR1000 datalogger. The relays were powered with two 10amp, 12v DC power supplies (Jameco Electronics, Belmont, CA).

One irrigation manifold supplied irrigation to each moisture treatment. The manifold was constructed of 1-1/4-inch o.d. PVC pipe, with the solenoid valve threaded into a 1/8-inch-1/8-inch union which was threaded into the manifold via a 1/8-27 NPT hole (Figure 15). Each

manifold had a 1-1/4"-1-1/4" coupling glued into each end, with a 1-1/4"-3/4" bushing glued into it. One end then had a 3/4-inch hose bib threaded into the bushing to drain the system. The opposite end had a 3/4" MNPT-5/8" garden hose adapter threaded into the bushing to connect the water supply.

The solenoid valves had a push-to-connect 1/8-inch-1/4-inch adapter joining it with the 1/4-inch blank microtubing (Rainbird, Azusa, CA). The irrigation tubing was terminated with a 1 gallon per hour, pressure compensating dripper emitter (Rainbird, Azusa, CA). The irrigation supply tube for each tube was secured through a 17/64-inch hole at the top of each growth tube (Figure 16). The dripper sat on the surface of the growth media. A parts list for the irrigation system is found in Appendix 1.

Nutrient solution was manually applied to the first experiment until the grain-fill drought stress was imposed in the drought treatment growth tubes. Afterward, water applied to either moisture treatment did not contain nutrient solution. This method resulted in lower fertility levels than desired. Therefore, nutrient solution was supplied with each irrigation through the automatic system in the second experiment. A 13 gallon Nalgene carboy, with spigot (United States Plastic Corp, Lima, OH) was connected to the irrigation manifolds via 5/8-inch garden hose. A 1.4 gallons per minute pump (Shurflo, Costa Mesa, CA) was controlled by the SDMCD16AC relay and transferred nutrient solution to irrigation manifold.

Peters 5-11-26 professional hydroponic nutrient solution (Hummert International, Earth City, MO) was mixed according to label instructions and supplemented with educational grade calcium nitrate (Fisher Scientific, Waltham, MA). Final nutrient concentrations in the liquid nutrient solution are found in (Table 4). These supplied concentrations were used in conjunction with the Osmocote Plus in the growth tubes to meet plant needs. The 5-11-26 solution was

completely dissolved at a 10x concentration in a 4000ml flask, then the calcium nitrate was added. The stock concentration was added to the carboy and brought to volume with reverse osmosis water supplied in the greenhouse.

Data collection programming

The Loggernet software package (Campbell Scientific, Logan, UT) was used for datalogger programming. The Shortcut program was initially used to construct basic commands for sensor query. The scan interval for data collection was set at every 6 hours. One CR1000 measured 6 multiplexers of soil moisture sensors, and the SDI 12 buss of MPS-6 sensors. The second CR1000 controlled the seventh multiplexer, and the three SDMCD16AC relays. The desired watering time determines the scan interval for the second CR1000, as it must be divisible by the scan interval. For the first experiment, a five minute scan interval was used, as there was no watering time less than five minutes. The plants required more water during the second experiment, and a one minute scan interval was used, allowing for more flexible watering times. The program used during the imposition of drought stress for CR1000 (1) is included as Appendix 2. The program used during the drought treatment for CR1000 (2) is included as Appendix 3.

Moisture treatment

The automatic watering program on CR1000(2) checked the readings from the top-depth EC-5 sensor in each tube, which were recorded on CR1000(1) and initiated irrigation at 8:00 a.m. if volumetric water content was found to be lower than the imposed thresholds. Until the drought stress was imposed, every tube was well-watered. During the first experiment, the well-watered irrigation threshold was 35% VWC at the top EC-5 sensor. Tubes with readings below this threshold received five minutes of irrigation, resulting in 189ml of supplied water. The

second experiment had greater plant development, and the threshold was set at VWC of 38%. This allowed the once-daily irrigation to maintain a well-watered condition throughout the day. . Because the growth media drains quickly and plants rapidly used water during the grain-fill period, more robust plants resulting from greater fertility management required a lower irrigation threshold or longer irrigation time to maintain a well-watered condition in experiment 2. When stress was imposed to the drought treatment tubes 10 days after head emergence, the growth tubes were irrigated at very low levels (1-2 minutes) when matric potential, determined by the MPS-6 sensor, was lower than -500kPa. The goal was to avoid imposing a sudden stress.. This allowed the plants to adjust to the stress, and for drought tolerance mechanisms to develop. Because osmotic adjustment is known to take around 14 days (Babu et al., 1999), the period of controlled stress allowed this to take place. During experiment 1, the objective was to maintain tubes at a soil matric potential (Ψ_m) greater than or equal to -500kPa at the top MPS-6 sensor. Each day during the 10 day moderation period, small volumes of water (37-112ml) were manually supplied once daily via the irrigation system to tubes with Ψ_m less than -500kPa. During the second experiment, the moderation period was maintained automatically with each tube <-500kPa receiving 75mL of nutrient solution at 8:00 a.m., 3:00 p.m., and 9:00 p.m. After ten days of controlled drought stress, no further water was added and each tube was allowed to develop drought stress naturally. The well-watered treatment tubes were consistently maintained with an irrigation threshold of 38% VWC, as discussed above, twice daily (8:00 a.m. and 9:00 p.m.) until physiological maturity.

Plant measurements

Plant measurements were initiated ten days after spike emergence, on moisture treatment day 0. Chlorophyll Index was measured with a Konica-Minolta SPAD 502+ Leaf Meter

(Spectrum Technologies, Aurora, IL). Measurements were recorded as an average of three to five sampled flag leaves per growth tube, which were measured at three points along the length of the leaf blade. Stomatal resistance was measured with an SC-1 leaf porometer (Decagon Devices, Pullman, WA) by sampling the base of 2-3 flag leaves per tube. All measurements were conducted on the adaxial surface of the leaf. Drought treatment tubes were measured again every two days until flag leaf senescence, and well-watered treatment tubes were measured every four days until the corresponding tube in the drought treatment had senesced.

Leaf water potential was measured with a Model 1000 pressure chamber (PMS Instrument, Albany, OR). Measurements were taken when each tube exhibited visible signs of drought stress. The onset of lower leaf stress in the drought treatment was marked by wilting, chlorosis, or senescence of the lower canopy, (Figure 17). Measurements were taken mid-morning in the drought treatment tube and the corresponding tube in the well-watered treatment. Three upper leaves were sampled at both stress points, and the two which were most visibly different were read simultaneously with the pressure chamber. For the lower canopy stress reading, F-1 leaves were used, and for the flag leaf stress reading, flag leaves were sampled. Two diverse leaves were read to conserve nitrogen gas by sampling the range of variability in an entry at once. Leaf water potential was noted by visible extrusion of water from the xylem under magnification. Leaves were rehydrated in distilled water for 4 hours at 4°C after leaf water potential readings. After rehydration they were blotted dry and frozen at -80°C until osmolality measurements could be taken. Osmolality was measured to estimate osmotic potential. Sap was extruded from thawed tissue using a glass rod inside a 1.5 ml test tube. Individual leaves were read when possible, to obtain 2-3 readings per tube. Readings were taken with a Model 5600 Vapro vapor pressure osmometer (Elitech, Logan, UT). Osmolality readings in mmol kg^{-1} from

the vapor pressure osmometer were converted to Atm for estimation of osmotic potential (Ψ_s) following the formula provided by Kirkham (2005) on page 306. Osmotic adjustment was taken as the difference between osmotic potential of the paired optimal and drought stressed entry, and averaged by genotype.

Grain fill duration was derived as the difference between flag leaf senescence and head emergence dates. At physiological maturity, the entire aboveground biomass was harvested from each tube and oven dried at 35°C for 48h. Plant height in experiment 1 was taken as an average of 5 primary tillers after drying as the length (cm) from the growth media surface to the base of the spike. Plant height in experiment 2 was measured as the same distance but before harvesting biomass. Biomass was taken immediately after removal from the drier. Number of spikes were counted and the spikes were retained after obtaining the biomass.

Rooting characteristics were observed by opening each tube after laying it on its side (Figure 18) and carefully brushing away growth media until the deepest root was identified. The tubes were placed in a wooden cradle designed to prevent the weight of the tube from resting on the sensors. Distance from the growth media surface to the deepest root was taken as rooting depth. Roots were extracted from the Profile material by placing the belowground biomass on a No. 12 single triangle seed cleaning sieve (Seedburo, Des Plaines, IL) and shaking until the Profile dropped through the screen. Remaining Profile was brushed away with a small paintbrush and roots were placed in a paper bag to be oven dried at 35°C for 72h Mass was recorded immediately after drying. For root volume analysis, the root mass was submerged in distilled water, gently agitated to remove remaining Profile particles stuck to the roots and then re-dried. Root volume by water displacement was measured with a homemade displacement vessel based on Archimedes' principle (Figure 19).

Data Analysis

Data are still being collected from experiment 2. A comprehensive analysis will be completed for data across experiments at a later date. For experiment 1, data were analyzed with SAS version 9.4 (SAS Institute, Cary, NC). In an analysis of variance, moisture treatment (n=2) and genotype (n=9) were treated as fixed effects. Residual degrees of freedom and experimental error with no further random terms were used as the denominator effects for the F-tests. Linear regression was completed for biomass using all genotype by moisture treatment observations. Significant terms were identified using backward selection with $p < 0.05$ followed by the MaxR selection in Proc Reg to build the regression models. Data were analyzed for all genotypes in the experiment, as well as the *Ae. geniculata* only. Comparison of moisture treatment by genotype effects were completed with simple contrasts, as well as by the use of ratios of the drought versus the well-watered responses. These relative responses compare the mean of each drought treatment genotype with the companion tubes in the well-watered treatment. As mentioned above, these tubes were paired across treatments by similar spike emergence date. In doing so, comparisons could be made across treatments with more similar phenologies.

Results

A table of simple statistics for all response variables is presented as Table 3-5. The mean, standard deviation, as well as minimum and maximum for all traits are shown by moisture treatment. Mean grain fill duration was 30 days in the drought treatment compared with 35 days in the well-watered treatment. Biomass had a similar minimum in both treatments, but the maximum was four grams higher in the well-watered treatment. Root traits (volume, mass, depth) had very similar means in both moisture treatments. The mean stomatal resistance by day showed very little trend during the drought stress period in the well-watered treatment, while the

drought treatment mean steadily increased throughout the 16 day period. Water potential values of the soil and sampled leaves are shown at the two visual stress stages, lower leaf stress, and flag leaf stress. The soil water potential, as measured by the MPS-6 sensor at all three depths, varied greatly when plants senesced. At senescence, the range of Ψ_m at the most shallow sensor depth ranged from -815 kPa to -3943 kPa. The mean value of Ψ_m at plant senescence in the drought treatment was -2182 kPa.

The results of an analysis of variance for select traits for both the complete experiment, as well as for the *Ae. geniculata* only, are presented in Table 3-6. Because tiller number, growth type, adaptation, and selection influence differ so greatly between *Ae. geniculata* and wheat, excluding the wheat makes it easier to compare the accessions of *Ae. geniculata* to each other and determine if genotypic differences are present. Many traits showed differences between moisture treatments and genotypes. Overall, there was almost no interaction between genotype and moisture treatment.

A linear regression for biomass for all entries across moisture treatments in experiment 1 is shown as Table 3-7. Ten factors were identified in a model explaining biomass, with an R^2 of 0.9812. Five variables were identified as having a positive effect on biomass (stomatal resistance, day 4; water applied; spike weight; plant height; and root mass). Five variables showed a negative effect on biomass (soil water potential at senescence; stomatal resistance, day 0 and 16; chlorophyll index, Day 8; average osmotic potential at flag leaf stress).

Physiological responses of entries are summarized in Table 3-8. Osmotic adjustment (OA) was calculated as the difference of the mean osmotic potential at flag leaf stress of the drought treatment minus the companion well-watered treatment, by genotype. Two entries (289 and TAM111) had a negative value for OA, while positive values ranged from 12kPa (entry 36)

to 552 kPa (entry 18). Relative values presented in the table are valuable in estimating the effect of the drought treatment. They were calculated as the ratio of drought treatment observation to well-watered observations, by genotype. Relative stomatal resistance ranged from 114.3% (entry 311) to 291.6% (entry 289). A relative value of 100% would indicate identical stomatal resistance across treatments, whereas a high relative value indicates higher resistance in drought. All parameters in Table 3-8 were tested in analysis of variance with genotype as a fixed effect. No significant differences were detected (data not shown), likely due to the high standard deviation and power from low replication.

Three entries had relative chlorophyll index values exceeding 100%, indicating a higher mean chlorophyll index at the final measurement in drought stress than in the well-watered treatment. Relative biomass ranged from 53% (entry 11) to 106.3% (entry 289). Two entries had a relative biomass exceeding 100% (entries 289, 307). Relative grain-fill duration ranged from 76.3% (entry 11) to 103.3% (entry 289). The contrasting physiological responses of entry 18, 36 and 311 are shown in Figure 20, along with Ψ_m at the three sensor depths at the time of plant senescence.

Select trait means for genotype by treatment are presented in Table 3-9. These values are the simple means for genotype by treatment, and are not paired comparison of companion tubes, as calculated for the data presented in Table 3-8.

Discussion

There are many challenges associated with identifying drought tolerance, particularly with a wild relative such as *Ae. geniculata*. With paired moisture treatments, drought tolerance could be indicated by statistical similarity between moisture treatments. In that case, a genotype exhibited similar performance despite drought stress. Because genetic yield improvement must always be

improved simultaneously, a genotype which produces additional biomass under well-watered conditions would also be desirable. However, a genotype in that case may not be statistically similar under drought stress, but may still possess drought tolerance. Leaf health and physiological responses are therefore important and should be evaluated simultaneously with plant responses like grain-fill duration, biomass, grain mass, and rooting traits. The difficulty in breeding for drought tolerance is not likely because it is too genetically complex, but rather that it is difficult to identify, and the target drought situation is not universal (Blum, 2011). Difficulty to identify drought tolerance can be because of confounding experimental conditions, as well as the number of ways in which a plant can exhibit drought tolerance. Evaluating data from as many plant and soil responses as possible will likely lead to the clearest approach to selecting for drought tolerance.

The preliminary experiments with *Ae. geniculata* identified putative drought tolerance, as well as susceptibility. Entry 289 was tested in the advanced screening because it showed a poor grain-fill duration under drought stress in the preliminary experiments (data not shown). Because the preliminary screenings only had a drought treatment, it was not possible to determine that entry 289 may simply have a shorter phenology than similar accessions, as suggested by relative grain-fill duration, and relative biomass in the advanced screening (Table 3-8). The accession did exhibit leaf stress under drought (Table 3-8), but had a very short days to senescence under both moisture treatments. Overall, the lack of a truly drought susceptible genotype made differentiation of the *Ae. geniculata* entries difficult.

There were significant genotypic differences detected (Table 3-6). However, there was a lack of a genotype by moisture treatment interaction for all traits, except day 12 stomatal resistance in the combined analysis, which could have been caused by a few factors. First, it is

possible that the drought stress did not succeed in imposing differences between moisture treatments. This would result in similar grain-fill duration across moisture treatments resulting in only genotypic differences. This is less likely, by the number of significant differences for moisture treatment from the analysis of variance (Table 3-6). Secondly, it could indicate that every genotype tested exhibited similar levels of drought tolerance. Based on a Tukey means separation (data not shown) for nearly all responses, this seems to be the more likely explanation. Data in Table 3-8 show near uniformly high levels of relative chlorophyll index, grain-fill duration, and biomass. Lack of genotype by moisture treatment interactions seems to have been caused by similar drought tolerance for all genotypes. Based on the origin of the genotypes tested, and the lack of a true susceptible check, this seems like a plausible explanation.

Despite the similarity of genotypes tested across moisture treatments, some interesting trends between genotypes were detected. The linear regression for biomass in Table 3-7 showed predictable results, with a combination of positive and negative effectors on biomass. The variables with the largest effects in the analysis were spike weight, root mass, and average osmotic potential at flag leaf stress. Root mass did not differ by moisture treatment, and only showed significant differences in the combined analysis for genotype. These results were expected, since root mass and spike mass are components of biomass. Increased osmotic potential at flag leaf stress having a negative effect on biomass is likely because the elevated OP occurred in the lower yielding drought stress treatment. The significant negative effect of a high day 0 stomatal resistance may indicate that some genotypes in the study maintained higher stomatal resistance throughout grain-fill, resulting in decreased photosynthesis and biomass (Cornic, 2000). Because biomass was measured in grams, many factors in this analysis are

shown to have a very small effect, despite their significant contribution the overall model (Table 3-7).

The data in Table 3-8 highlight some of the most notable trends from experiment 1. The OA in the present study ranged from 12 kPa to 552 kPa (Table 3-8). These levels were consistent with prior reports of OA levels in *Ae. geniculata* (Mguis et al., 2012). OA has been a known strategy in wheat for some time (Morgan and Condon, 1986; Babu et al., 1999; Zhang et al., 1999; Blum et al, 1999; Blum, 2011) and literature has suggested that OA is present in *Ae. geniculata* through screening with poly ethylene glycol (Reikka et al., 1998a; Reikka et al., 1998b; Farooq 2001; Mguis et al., 2012) . OA is an important component of drought tolerance, and has not been shown to affect yield potential by compromising other plant processes (Blum, 2005). The accompanying data on relative stomatal resistance, chlorophyll index, biomass, and grain-fill duration may help clarify potential varying strategies in the *Ae. geniculata*. It was reported by Mguis et al. (2013) that stomatal resistance in stress varied in *Ae. geniculata*, largely by geographic origin. Variation was seen in stomatal resistance (Table 3-5, Table 3-8), with measurements late in the moisture treatment period (Day 12, 16) being significantly different between moisture treatment, and Day 12 showing genotypic variation in the *Ae. geniculata* (Table 3-6). These measurements with the leaf porometer were difficult to obtain due to small leaf area (data not shown), and additional variation was observed between cloudy and sunny days (personal observation- data not shown). Leaf size of the *Ae. geniculata* also made measurements difficult with the porometer.

The role of ABA signaling in drought stress suggests that there may be multiple tolerance strategies present in the tested germplasm. ABA signaling acts as an early warning signal (Blum, 2011), and the plant often responds with restricted growth (Ali et al., 1999; Sreedhar et al.,

2002), deeper rooting (Morgan and Condon, 1986; Munns and Sharp, 1993), reduced phenology (Ali et al., 1999), reduced stomatal conductance (Ali et al., 1999; Blum, 2011), accelerated leaf senescence (Munne-Bosch and Alegre, 2004), and osmotic adjustment (Ali et al., 1999). Thus, elevated levels of ABA may lead to increased stomatal resistance, decreased chlorophyll index (SPAD), and shorter grain-fill duration. The high OA, but lower relative chlorophyll index, and high relative stomatal resistance suggest that entry 18 may be overly sensitive to ABA signaling, leading to its lower relative biomass from a premature photosynthetic shutdown (Table 3-8). It may be exhibiting isohydric behavior, which would be expected due to origin in the Mediterranean (Blum, 2015). Alternatively, entry 311 had the next highest OA (438 kPa), with the lowest relative stomatal resistance and highest relative chlorophyll index (Table 3-8). This genotype appears to adjust to the lower leaf water potential, and maintain higher levels of photosynthesis and biomass production. Its lower relative biomass and grain-fill duration were mainly a reflection of its high biomass and grain-fill duration in the well-watered treatment (Table 3-9), as well as one pair of companion tubes being very disproportionate (data not shown). Based on this data, it appears to be exhibiting anisohydric behavior, more compatible with a strong osmotic adjustment and continued growth and water use (Blum, 2015). This type of drought tolerance is more compatible with crop production, because available water is effectively used, and stomatal closure does not prevent photosynthesis (Blum, 2015). This opportunistic growth in well-watered conditions is also a more ideal response for crop plants. Entry 311 (TA10437) has been previously noted for its superior phenotype (personal observation) and is the source of the *Lr57/Yr40* gene in wheat (Aghaee-Sarbarzeh et al., 2002; Kurupathy et al., 2007). Both entry 18 and entry 311 senesced at very low soil moisture levels (Figure 20).

Visual observations of response to drought are important, in addition to holistic evaluation of plant responses. Because smaller statured plants may exhibit higher drought tolerance (Blum et al., 1997), careful evaluation of germplasm sources which may be compatible with grain production in crop plants is important. Drought treatment conditions must also be tailored to specific environmental conditions, because available water in the soil profile and seasonal precipitation will dictate what type of stress response is most desirable (Blum, 2015). The advanced screening system is advantageous for further drought screening because of how it can be customized to screen for differing stress types by carefully manipulating available water.

In conclusion, there appear to be varying modes of tolerance to drought stress despite the statistical similarity between genotypes across moisture treatments. Further follow-up with the incorporation of data from experiment 2 to confirm these results will provide additional evidence. Further physiological characterization, such as measuring ABA, may provide additional evidence to support the emerging theories on the different responses. Hybridization of the most desirable genotypes with wheat may provide desirable diversity beyond drought tolerance, and the progeny of such a cross could be evaluated for response with this screening system to study the effect of the *Ae. geniculata* genes in a wheat background. This system also represents a great resource to study other wild relatives which may possess even greater levels of drought tolerance, such as *Triticum turgidum subsp. dicoccoides* (Reikka et al., 1998 a and b), *Ae. cylindrica*, and *Ae. tauschii* (Farooq, 2001). This system also allows for intensive screening of a limited number of wheat genotypes for drought stress while minimizing all other sources of variation.

Table 3-1 Genotypes and Their Sources Screened in the first Preliminary *Aegilops geniculata* Drought Screening Experiment

KSU Id #	Genotype	Source	KSU Id #	Genotype	Source	KSU Id #	Genotype	Source
1	CIae 43	NSGC†	32	PI374365	NSGC	76	PI542190	NSGC
4	CIae 65	NSGC	33	PI374374	NSGC	77	PI551083	NSGC
7	PI170195	NSGC	34	PI388754	NSGC	78	PI551084	NSGC
8	PI170206	NSGC	35	PI388755	NSGC	79	PI551085	NSGC
11	PI276978	NSGC	36	PI388756	NSGC	81	PI551087	NSGC
13	PI289578	NSGC	37	PI483009	NSGC	82	PI551089	NSGC
14	PI298899	NSGC	38	PI483022	NSGC	83	PI551090	NSGC
15	PI330487	NSGC	50	PI487227	NSGC	84	PI551091	NSGC
17	PI361880	NSGC	51	PI487228	NSGC	85	PI551092	NSGC
18	PI361881	NSGC	53	PI487283	NSGC	89	PI551096	NSGC
19	PI369574	NSGC	55	PI491426	NSGC	90	PI551098	NSGC
20	PI369575	NSGC	56	PI491427	NSGC	91	PI551100	NSGC
21	PI369576	NSGC	62	PI491433	NSGC	93	PI551102	NSGC
22	PI369577	NSGC	64	PI524953	NSGC	95	PI551104	NSGC
23	PI369578	NSGC	65	PI524954	NSGC	98	PI551107	NSGC
24	PI369579	NSGC	67	PI524956	NSGC	101	PI551110	NSGC
25	PI369580	NSGC	68	PI542180	NSGC	102	PI551111	NSGC
26	PI374324	NSGC	70	PI542182	NSGC	103	PI551112	NSGC
27	PI374337	NSGC	71	PI542185	NSGC	104	PI551113	NSGC
28	PI374338	NSGC	72	PI542186	NSGC	108	PI551117	NSGC
29	PI374339	NSGC	73	PI542187	NSGC	109	PI551118	NSGC
31	PI374361	NSGC	75	PI542189	NSGC	111	PI554277	NSGC
Continued on next page								

† National Small Grains Collection, Aberdeen, ID

KSU Id #	Genotype	Source	KSU Id #	Genotype	Source	KSU Id #	Genotype	Source
112	PI554278	NSGC	159	PI573398	NSGC	253	TA2240	WGRC
114	PI554280	NSGC	160	PI573399	NSGC	254	TA2241	WGRC
115	PI554281	NSGC	162	PI573401	NSGC	262	TA2255	WGRC
116	PI554284	NSGC	163	PI573402	NSGC	263	TA2256	WGRC
122	PI564184	NSGC	165	PI573404	NSGC	266	TA2649	WGRC
123	PI564185	NSGC	166	PI573405	NSGC	267	TA2650	WGRC
124	PI564186	NSGC	171	PI573410	NSGC	268	TA2651	WGRC
125	PI564187	NSGC	175	PI614626	NSGC	270	TA2653	WGRC
127	PI564190	NSGC	180	TA1721	WGRC [‡]	274	TA2899	WGRC
128	PI564191	NSGC	183	TA1799	WGRC	276	TA10003	WGRC
133	PI573372	NSGC	194	TA1811	WGRC	278	TA10005	WGRC
134	PI573373	NSGC	196	TA1813	WGRC	279	TA10006	WGRC
135	PI573374	NSGC	200	TA1817	WGRC	280	TA10008	WGRC
137	PI573376	NSGC	202	TA1819	WGRC	281	TA10009	WGRC
138	PI573377	NSGC	207	TA2041	WGRC	283	TA10012	WGRC
139	PI573378	NSGC	211	TA2046	WGRC	285	TA10015	WGRC
140	PI573379	NSGC	213	TA2048	WGRC	286	TA10018	WGRC
141	PI573380	NSGC	215	TA2051	WGRC	288	TA10020	WGRC
142	PI573381	NSGC	218	TA2058	WGRC	289	TA10021	WGRC
146	PI573385	NSGC	232	TA2165	WGRC	290	TA10022	WGRC
147	PI573386	NSGC	235	TA2185	WGRC	291	TA10023	WGRC
148	PI573387	NSGC	236	TA2187	WGRC	292	TA10024	WGRC
149	PI573388	NSGC	237	TA2188	WGRC	302	TA10036	WGRC
152	PI573391	NSGC	239	TA2191	WGRC	303	TA10037	WGRC
155	PI573394	NSGC	242	TA2221	WGRC	304	TA10038	WGRC
156	PI573395	NSGC	243	TA2222	WGRC	307	TA10041	WGRC
157	PI573396	NSGC	244	TA2223	WGRC	311	TA10437	WGRC
158	PI573397	NSGC	251	TA2238	WGRC	-	TA2899	WGRC

† National Small Grains Collection, Aberdeen, ID

‡ Wheat Genetics Resource Center, Manhattan, KS

Table 3-2 Genotypes and their Source Screened in the Second Preliminary Drought Experiment

KSU ID	Genotype	Source	KSU ID	Genotype	Source	KSU ID	Genotype	Source
1	CIae 43	NSGC†	101	PI551110	NSGC	239	TA2191	WGRC
4	CIae 65	NSGC	102	PI551111	NSGC	242	TA2221	WGRC
8	PI170206	NSGC	103	PI551112	NSGC	243	TA2222	WGRC
11	PI276978	NSGC	104	PI551113	NSGC	244	TA2223	WGRC
17	PI361880	NSGC	108	PI551117	NSGC	251	TA2238	WGRC
18	PI361881	NSGC	115	PI554281	NSGC	262	TA2255	WGRC
22	PI369577	NSGC	122	PI564184	NSGC	266	TA2649	WGRC
23	PI369578	NSGC	123	PI564185	NSGC	267	TA2650	WGRC
25	PI369580	NSGC	124	PI564186	NSGC	270	TA2653	WGRC
26	PI374324	NSGC	127	PI564190	NSGC	274	TA2899	WGRC
29	PI374339	NSGC	133	PI573372	NSGC	276	TA10003	WGRC
34	PI388754	NSGC	134	PI573373	NSGC	278	TA10005	WGRC
35	PI388755	NSGC	135	PI573374	NSGC	279	TA10006	WGRC
36	PI388756	NSGC	141	PI573380	NSGC	280	TA10008	WGRC
50	PI487227	NSGC	148	PI573387	NSGC	281	TA10009	WGRC
53	PI487283	NSGC	149	PI573388	NSGC	283	TA10012	WGRC
62	PI491433	NSGC	156	PI573395	NSGC	285	TA10015	WGRC
65	PI524954	NSGC	158	PI573397	NSGC	286	TA10018	WGRC
68	PI542180	NSGC	165	PI573404	NSGC	288	TA10020	WGRC
70	PI542182	NSGC	180	TA1721	WGRC‡	289	TA10021	WGRC
72	PI542186	NSGC	194	TA1811	WGRC	290	TA10022	WGRC
73	PI542187	NSGC	196	TA1813	WGRC	292	TA10024	WGRC
75	PI542189	NSGC	200	TA1817	WGRC	302	TA10036	WGRC
76	PI542190	NSGC	202	TA1819	WGRC	303	TA10037	WGRC
78	PI551084	NSGC	207	TA2041	WGRC	304	TA10038	WGRC
81	PI551087	NSGC	215	TA2051	WGRC	307	TA10041	WGRC
90	PI551098	NSGC	232	TA2165	WGRC	311	TA10437	WGRC
91	PI551100	NSGC	235	TA2185	WGRC	-	2899	WGRC
93	PI551102	NSGC	236	TA2187	WGRC	-	TAM111	Check
98	PI551107	NSGC	237	TA2188	WGRC	-	TAM112	Check

† National Small Grains Collection, Aberdeen, ID

‡ Wheat Genetics Resource Center, Manhattan, KS

Table 3-3 Genotypes used in Advanced Drought Screening, with Source and Collection Information

Entry ID	Genotype	Source	Species	Nearest City to Collection Site	Country of Origin
11	PI 276978	NSGC†	<i>Aegilops geniculata</i>	Unknown	Unknown
18	PI 361881	NSGC	<i>Aegilops geniculata</i>	Cluj	Romania
36	PI 388756	NSGC	<i>Aegilops geniculata</i>	Moulay-Bouazza	Morocco
289	TA10021	WGRC‡	<i>Aegilops geniculata</i>	Ezzhiliga (Rabat)	Morocco
307	TA10041	WGRC	<i>Aegilops geniculata</i>	Essaouira	Morocco
311	TA10437	WGRC	<i>Aegilops geniculata</i>	Unknown	Republic of Kosovo
TAM 111		Check	<i>Triticum aestivum</i>	-	United States of America
TAM 112		Check	<i>Triticum aestivum</i>	-	United States of America
Santa Fe		Check	<i>Triticum aestivum</i>	-	United States of America

† National Small Grains Collection, Aberdeen, ID

‡ Wheat Genetics Resource Center, Manhattan, KS

Table 3-4 Final Nutrient Concentrations in Nutrient Solution Applied through Irrigation

		ppm
Nitrate	N	150
Phosphorous	P	48
Potassium	K	216
Calcium	Ca	116
Magnesium	Mg	31
Sulfate	SO ₄	125
Iron	Fe	3
Manganese	Mn	0.5
Zinc	Sn	0.15
Copper	Cu	0.15
Boron	B	0.5
Molybdenum	Mo	0.1

Table 3-5 Simple Statistics for *Ae. geniculata* Entries in Drought Experiment 1 only, by Moisture Treatment

	Unit	Well-Watered				Drought			
		Mean	Std Dev	Min	Max	Mean	Std Dev	Min	Max
Grain-Fill Duration	Days	35	7	21	45	30	3	24	35
Biomass		9	3	5	13	7	1	5	9
Spike Weight	g	5	2	2	7	4	1	2	5
Root Mass		6	2	4	9	5	1	4	7
Plant Height	cm	42	13	25	56	34	9	20	46
Root Depth		70	17	50	114	72	17	49	101
Root Volume	cm ³	6	2	3	10	6	2	2	9
Water Applied		3812	1314	1577	5363	943	349	442	1451
Stomatal Resistance, Day 0		768	255	491	1345	1002	283	593	1519
Stomatal Resistance, Day 4		1019	328	518	1472	900	306	472	1366
Stomatal Resistance, Day 8	s m ¹	940	307	533	1356	1237	849	562	3646
Stomatal Resistance, Day 12		807	241	474	1158	1701	975	646	3503
Stomatal Resistance, Day 16		1223	362	684	1905	1913	505	1021	2623
Chlorophyll Index, Day 0		46	4	40	51	46	3	41	50
Chlorophyll Index , Day 4		45	4	36	50	44	5	31	52
Chlorophyll Index , Day 8		44	5	35	50	43	5	36	50
Chlorophyll Index , Day 12		43	4	36	47	41	6	31	49
Chlorophyll Index , Day 16		43	5	32	48	39	7	22	44
LWP [†] at lower canopy stress		-1581	413	-2400	-1075	-2743	1610	-6000	-1500
30.5 cm SWP [‡] ; lower canopy stress		-2	1	-4	-1	-883	511	-1794	-292
LWP, flag leaf stress	kPa	-1552	291	-2000	-1050	-2936	1381	-6000	-1200
30.5 cm SWP; flag leaf stress		-2	1	-4	-1	-2176	1208	-4836	-650
61 cm SWP; flag leaf stress		-3	1	-4	-2	-244	318	-899	-19
91.5 cm SWP; flag leaf stress		-3	1	-5	0	-11	5	-21	-8
Osmotic Potential, Flag Leaf Stress		1892	197	1617	2197	2026	237	1745	2602
30.5 cm SWP at senescence		-8	17	-63	-1	-2182	955	-3943	-815
61 cm SWP at senescence		-7	10	-38	-2	-226	282	-795	-18
91.5 cm SWP at senescence		-3	1	-5	-1	-10	3	-19	-9

Table 3-6 Analysis of Variance p-value Significance Results for Select Response Variables in Experiment 1 for *Ae. geniculata* only, and Combined Analysis of all Entries for Fixed Effects of Moisture Treatment, Genotype, and their Interaction

	Moisture Treatment		Genotype		Genotype* Treatment	
	<i>Ae. geniculata</i>	All	<i>Ae. geniculata</i>	All	<i>Ae. geniculata</i>	All
Spike Weight	NS	NS	NS	NS	NS	NS
Grain Fill Duration	***	***	***	***	NS	NS
Biomass	**	**	*	**	NS	NS
Height	***	NS	***	**	NS	NS
Root Mass	NS	NS	NS	**	NS	NS
Root Depth	NS	NS	NS	NS	NS	NS
Stomatal Resistance, Day 12	***	***	**	NS	NS	*
Stomatal Resistance, Day 16	***	**	NS	.	NS	NS
Chlorophyll Index, Day 12	NS	NS	***	**	NS	NS
Chlorophyll Index, Day 16	NS	NS	*	NS	NS	NS
Soil Water Potential at Flag Leaf Senescence	***	***	NS	NS	NS	NS
Leaf Water Potential at Flag Leaf Wilting	*	**	NS	NS	NS	NS
Osmotic Potential at Flag Leaf Wilting	NS	NS	NS	NS	NS	NS

*p<0.05; **p<0.01; ***p<0.001

Table 3-7 Significant Factors in a Linear Regression Model for Biomass of both Moisture Treatments, with all Entries in Experiment 1, Maximized for R²

Variable	Est.	S.E.	Pr > F
Intercept	10.9823	2.14	0.0002
Soil Water Potential at Senescence	-0.0007	0.00	0.0005
Stomatal Resistance, Day 0	-0.0013	0.00	0.01
Chlorophyll Index, Day 8	-0.1390	0.02	0.0001
Stomatal Resistance, Day 16	-0.0010	0.00	0.0022
Water Applied	0.0005	0.00	0.0006
Spike Weight	0.9504	0.16	<.0001
Plant Height	0.0320	0.01	0.0241
Root Mass	0.4214	0.07	<.0001
Osmotic Potential at Flag Leaf Stress	-0.2339	0.07	0.0076

R² =0.9812

Table 3-8 Mean Physiological Responses of Genotypes in Experiment 1. Mean Osmotic Adjustment; Relative Final Stomatal Resistance, Chlorophyll Index, Biomass, and Grain-Fill Duration

Genotype	OA	Rel. Stomatal Resistance	Rel. Chlorophyll Index	Rel. Biomass	Rel. Grain Fill Duration
	(kPa)				
11	52	274.5%	96.5%	53.0%	76.3%
18	552	250.4%	83.0%	61.1%	92.4%
36	12	179.5%	100.0%	93.7%	78.6%
289	-137	291.6%	60.3%	106.3%	103.3%
307	213	184.5%	85.6%	105.2%	87.9%
311	438	114.3%	118.6%	82.0%	77.0%
TAM 111	-46	173.4%	68.1%	81.0%	92.2%
TAM 112	211	135.6%	137.4%	100.7%	91.2%
SantaFe	400	186.9%	88.4%	91.6%	93.6%

Table 3-9 Mean of Select Genotypic Responses, by Moisture Treatment for Experiment 1

Moisture Treatment	Genotype	GFD	Water Applied	Biomass	Spike Weight	Height	Root Depth	Root Mass	Root Volume	Osmotic Potential (Flag Leaf Stress)
		Days	cm ³	g	g	cm	cm	g	cm ³	kPa
Drought	11	29	883	6.2	3.5	37.3	68.0	6.9	4.3	1953.5
Well-Watered	11	37.5	4732	12.55	5.7	56.0	75.0	7.1	6.2	2005.0
Drought	18	30.5	946	7.1	3.9	40.4	67.0	5.1	7.8	2173.3
Well-Watered	18	33	4416	11.45	6.3	48.5	82.0	7.4	10.0	1936.1
Drought	36	31.5	662	7.9	4.9	35.5	75.0	5.7	7.3	1840.4
Well-Watered	36	40.5	3785	8.7	4.8	34.5	61.0	6.0	7.0	1828.0
Drought	289	24	678	6.15	3.6	20.5	62.8	4.7	5.1	1973.4
Well-Watered	289	23.5	1735	5.85	2.7	25.5	64.5	5.0	6.2	2110.8
Drought	307	34	1293	6.15	4.2	28.5	87.5	5.5	4.5	2100.2
Well-Watered	307	39	3470	5.85	3.6	32.5	78.0	4.0	4.5	1887.1
Drought	311	30	1167	8.35	4.4	42.8	71.0	4.4	4.5	2078.5
Well-Watered	311	39	4732	10.35	5.2	53.5	61.0	4.1	4.7	1640.6
Drought	Santa Fe	29.5	726	6.05	3.5	50.0	51.0	6.1	6.1	2402.2
Well-Watered	Santa Fe	31.5	3312	6.7	4.0	51.7	65.8	4.7	8.3	2002.0
Drought	TAM 111	27	410	7.5	4.2	55.7	54.0	6.3	7.5	2173.5
Well-Watered	TAM 111	29.5	2997	9.2	5.0	65.8	89.0	5.0	6.7	2219.1
Drought	TAM 112	30.5	662	5.85	3.5	49.2	71.0	6.3	5.4	2545.3
Well-Watered	TAM 112	32.5	2366	5.95	3.7	47.3	52.0	3.8	6.8	2333.8
CV	Drought	9.9	45.0	20.5	23.3	28.8	27.0	18.3	34.6	13.4
	Well-Watered	17.3	34.1	31.1	28.3	28.3	26.6	31.4	32.6	15.5

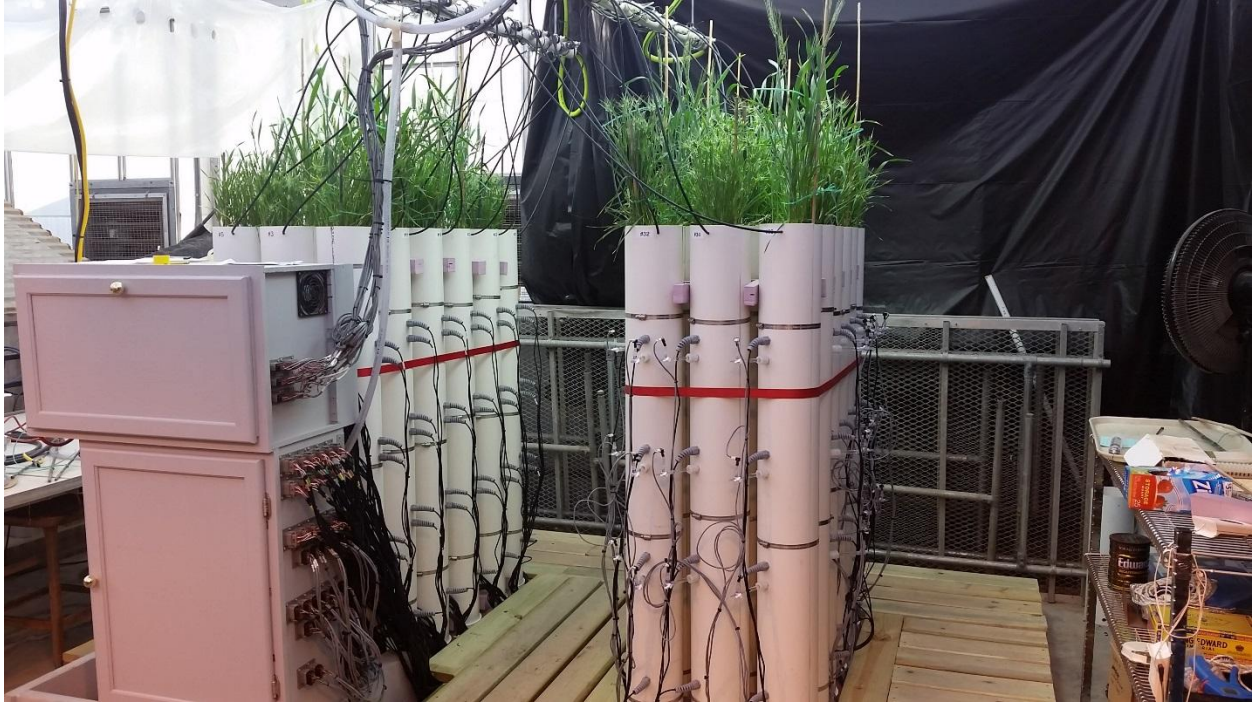


Figure 3-1 The Entire Experimental Setup of the Drought Screening System

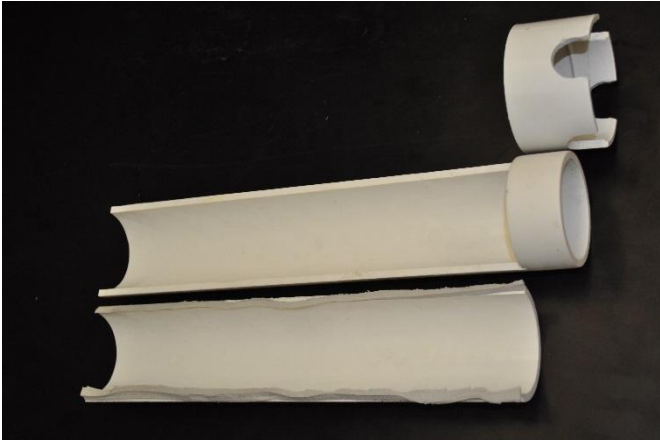


Figure 3-2 (clockwise) A View of the Split Tubes, Partially Filled with Sensors; Disassembled 1:10 Scale Model of Tube Showing Cuts in PVC pipe; Drain Components in Order of Assembly



Figure 3-3 The Drill Press Jig to Securely Drill Pilot Holes in One Half of the Tubes, for Sensor Placement.

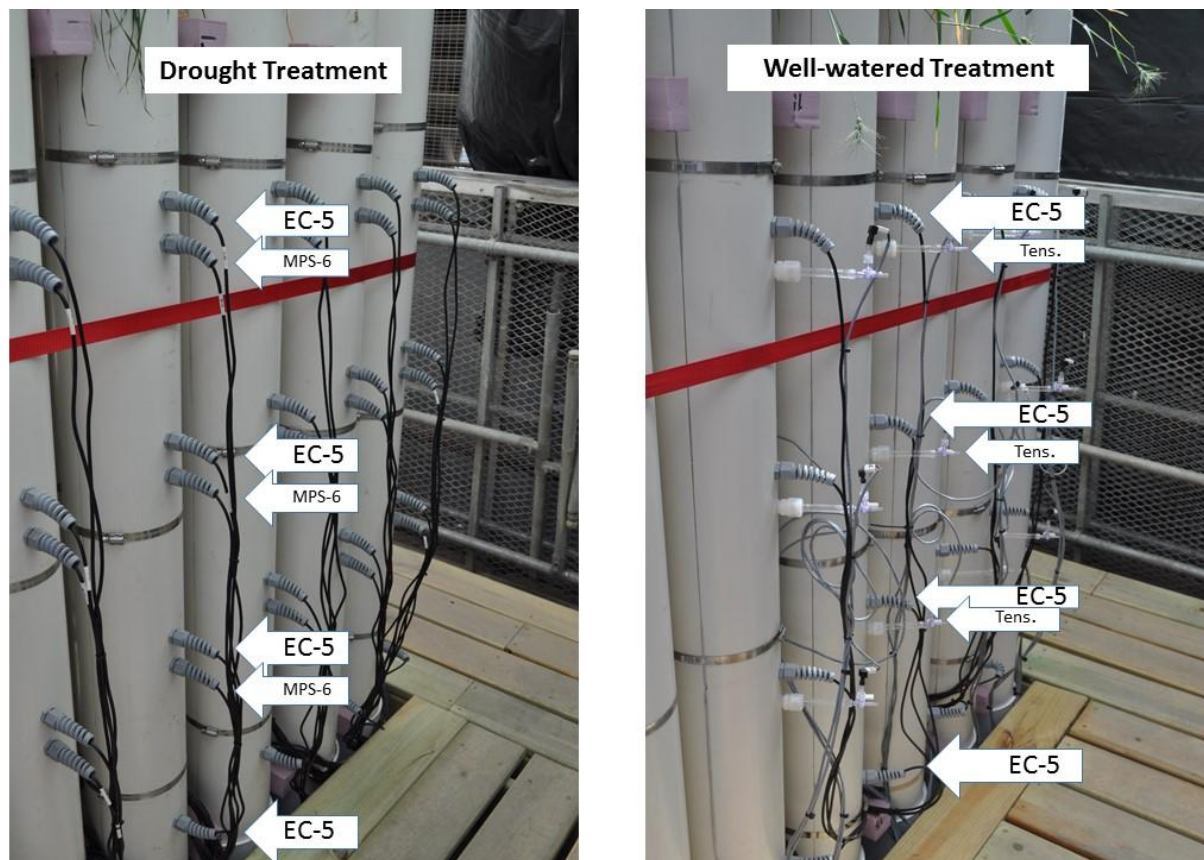


Figure 3-4 Sensor Placement in two Types of Moisture Treatment Tubes in Advanced Drought Screening



Figure 3-5 The Assembled Calibration Tube, Immediately After Saturation

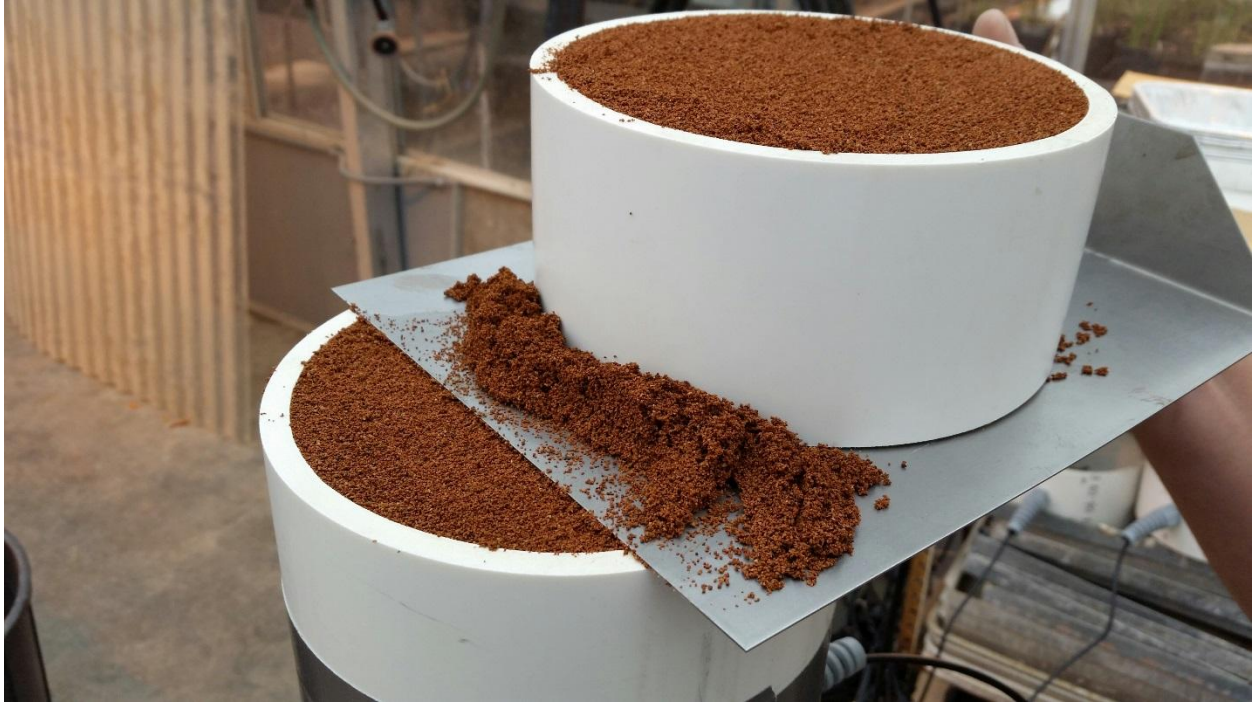


Figure 3-6 Sectioning the Calibration Tube for Wet Mass Measurement

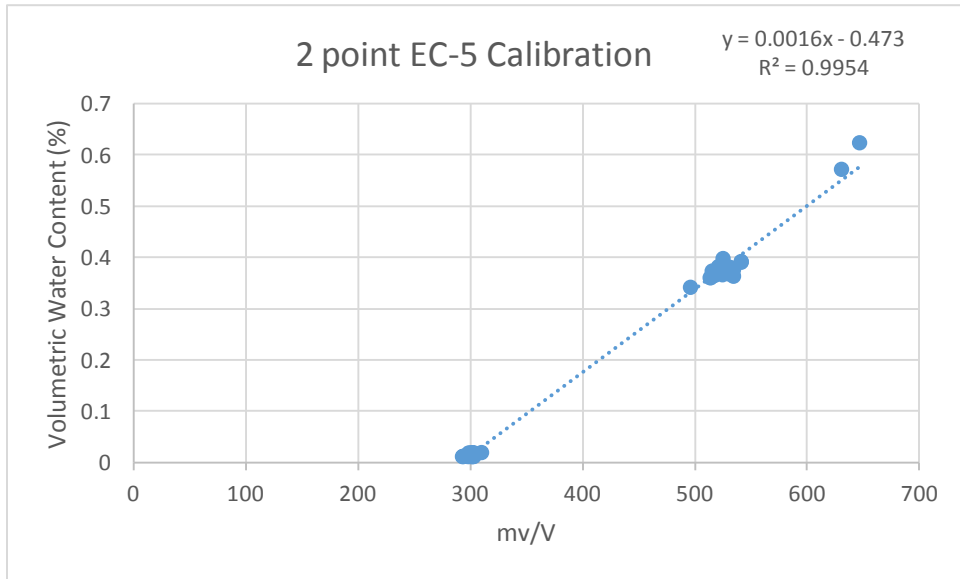


Figure 3-7 EC-5 Calibration Curve Showing Regression of Volumetric Water Content (%) on Millivolts Volts-1 Sensor Output; $R^2=0.9954$

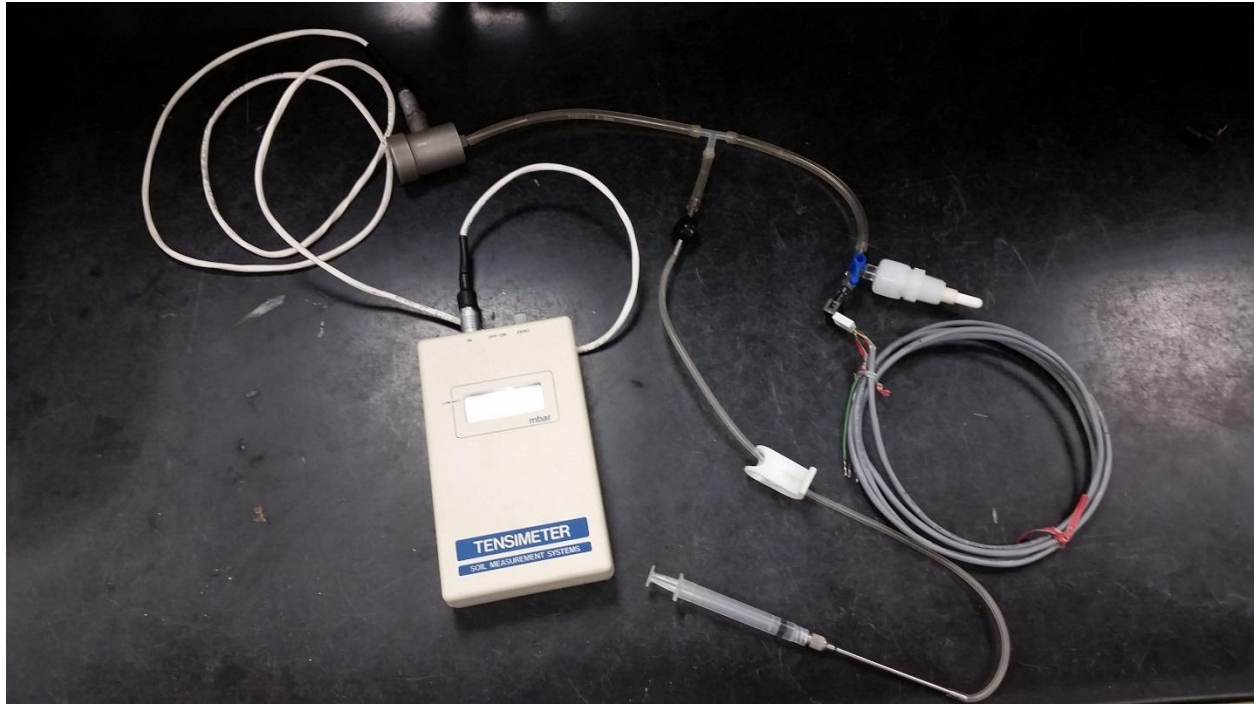


Figure 3-8 The Tensimeter, Syringe, and Tygon Tubing Apparatus Used for Calibration of Pressure Transducers, Shown Connected to a Different Style of Mini-Tensiometer for Example

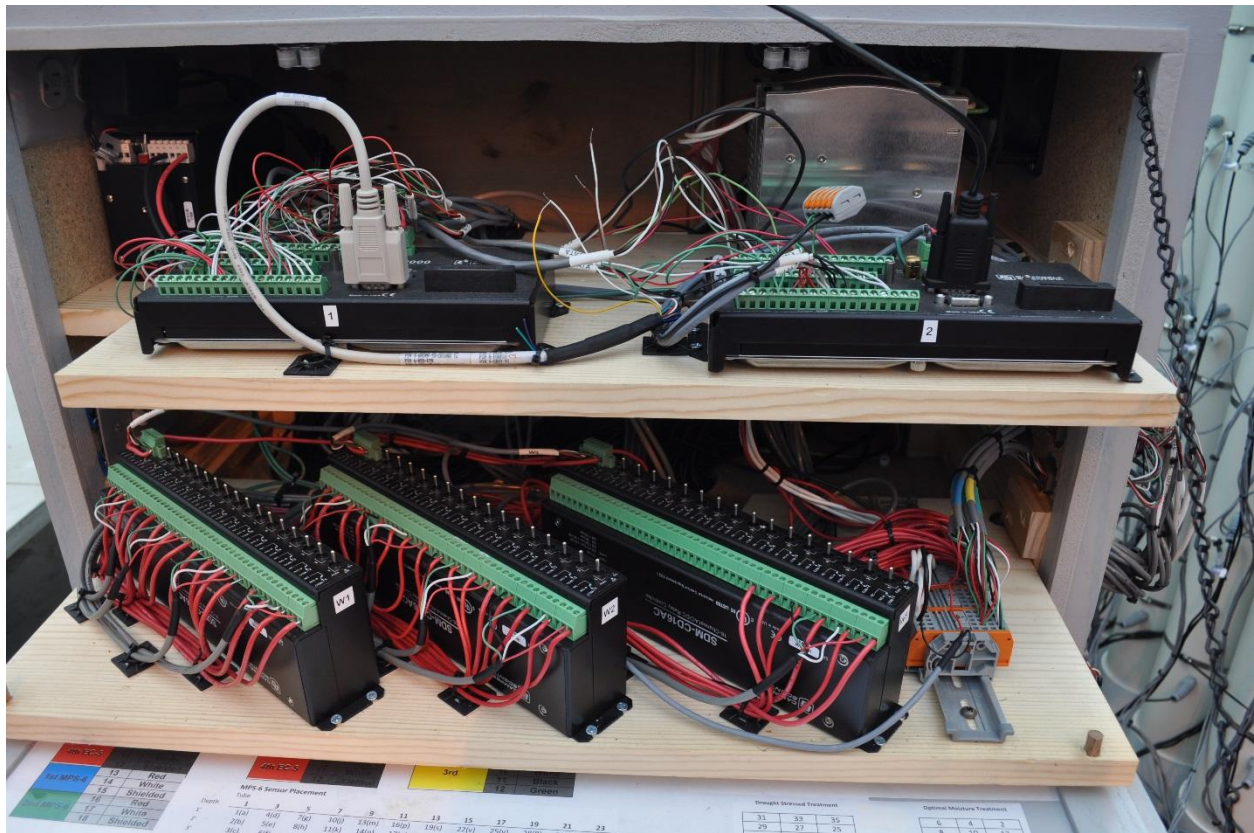


Figure 3-9 The two CR1000 Dataloggers (top shelf), with 10amp DC Power Supply Visible in rear, and SDMCD16AC Relays Powering Irrigation System (bottom shelf)

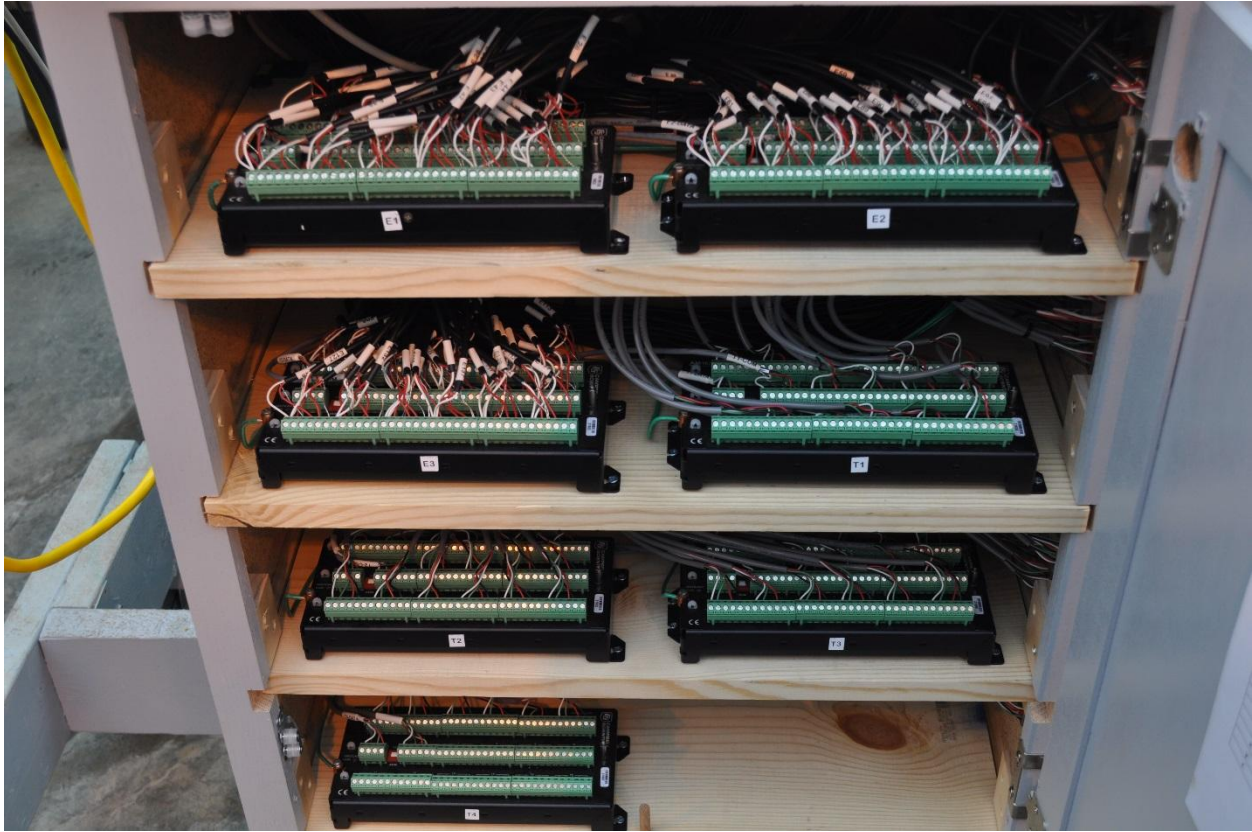


Figure 3-10 Seven AM1632B Multiplexers in Data Acquisition Cabinet

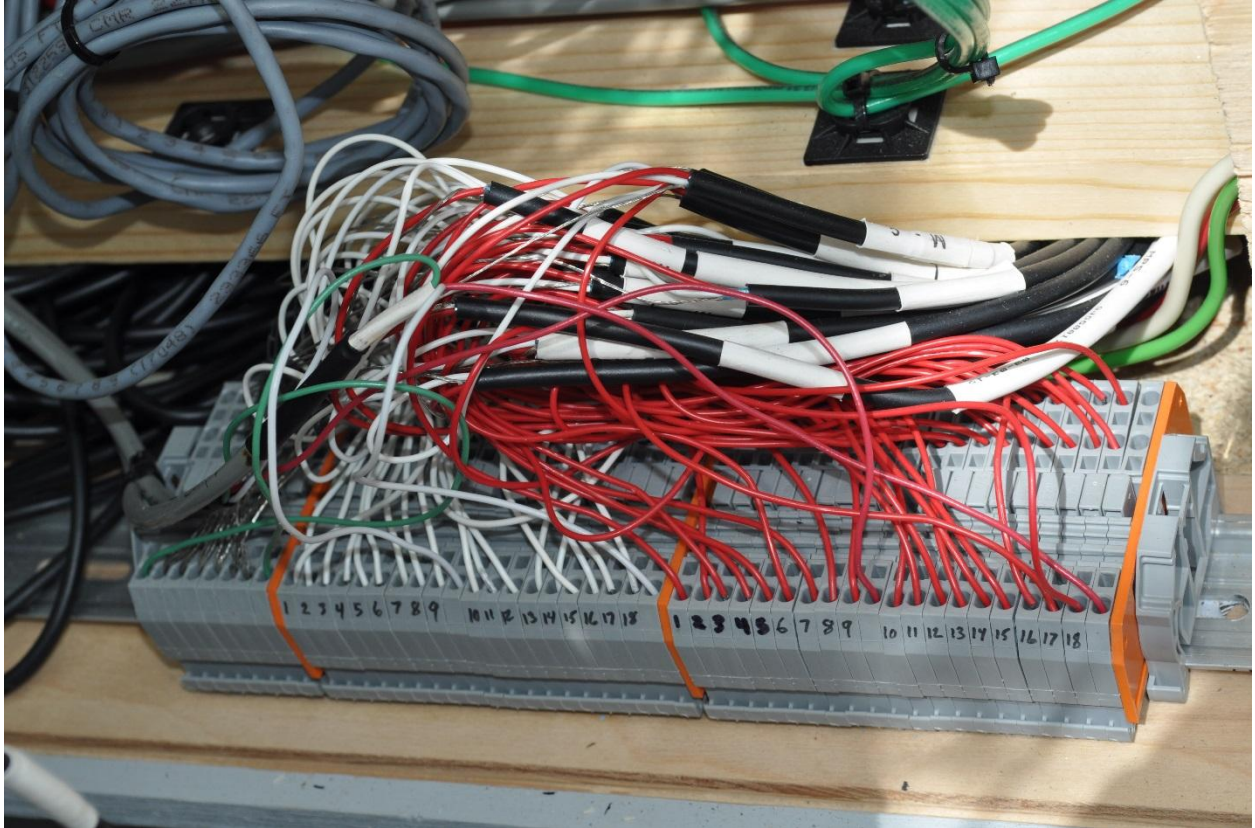
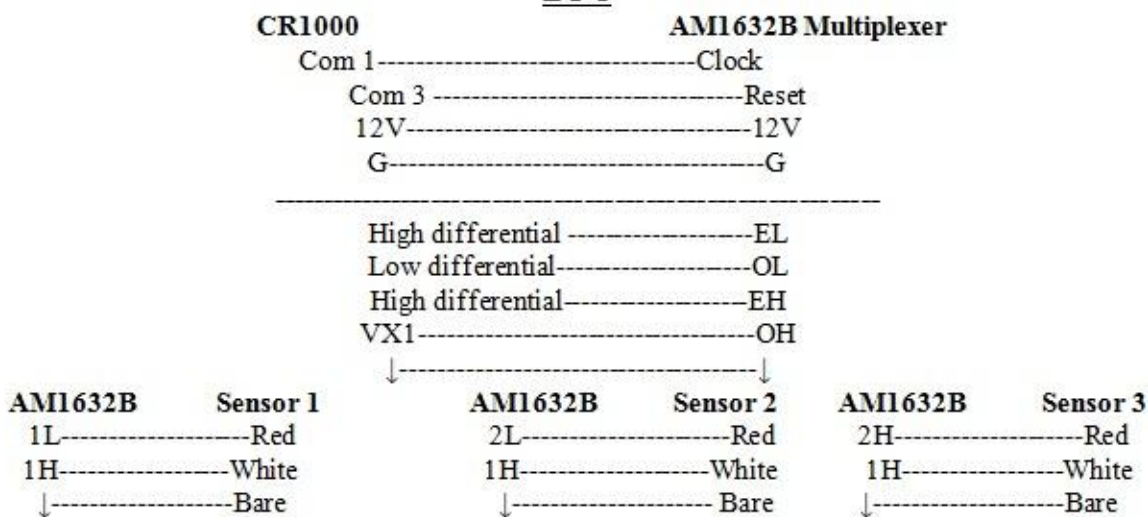
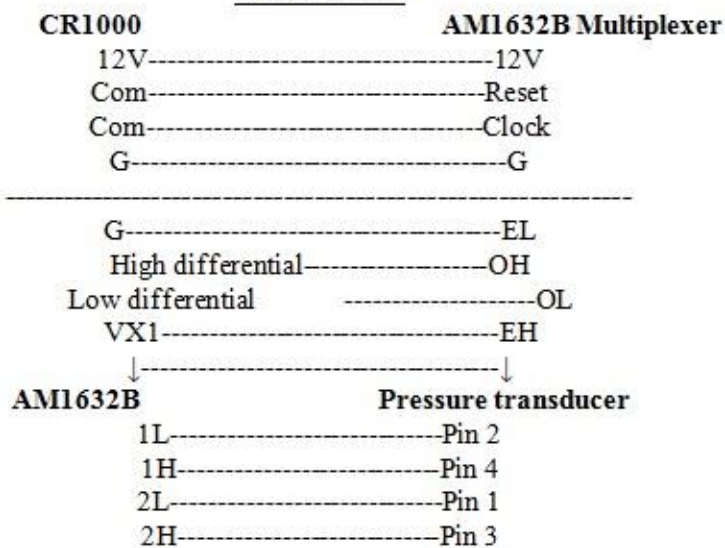


Figure 3-11 DIN Rail Terminal Assembly for Terminating 54 MPS-6 Matric Potential Sensors

EC-5



Tensiometers



SDMCD16AC Irrigation Relays

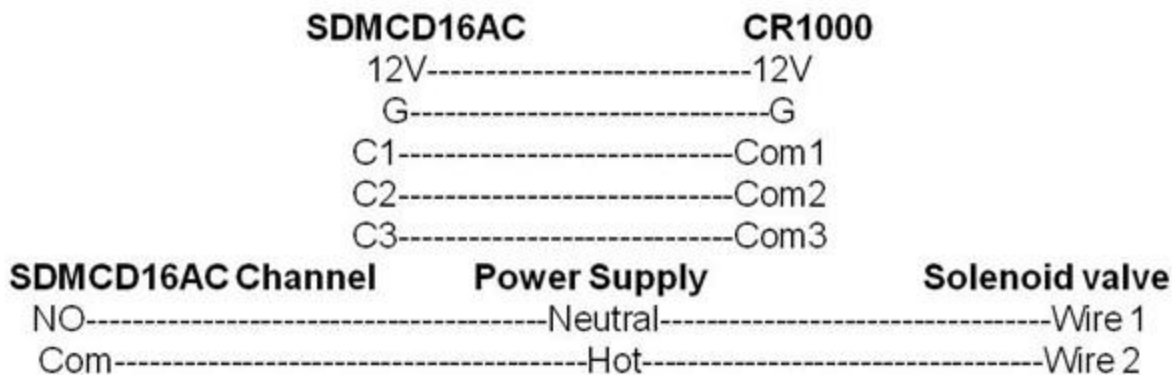


Figure 3-12 Wiring Diagram Examples for CR1000 Dataloggers

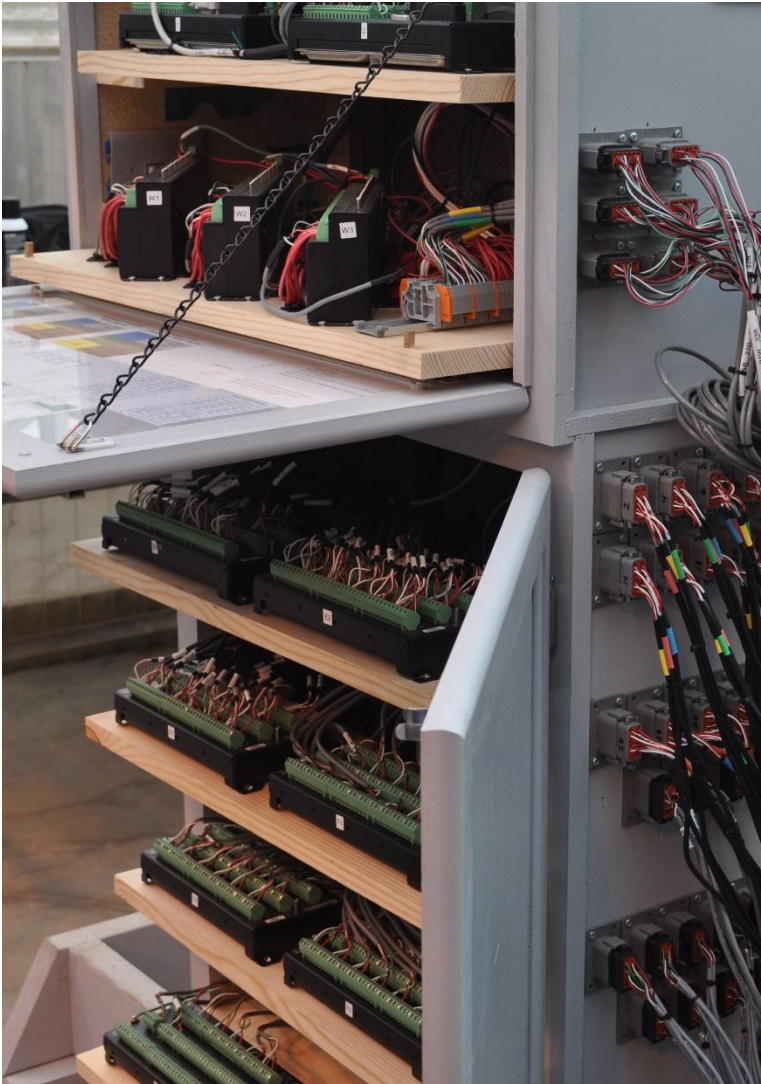


Figure 3-13 Data Acquisition Cabinet



Fig used for Sensors in Drought Treatment Tubes (left) and 12 pin sensors in Well-Watered Tubes, and Irrigation System (right).

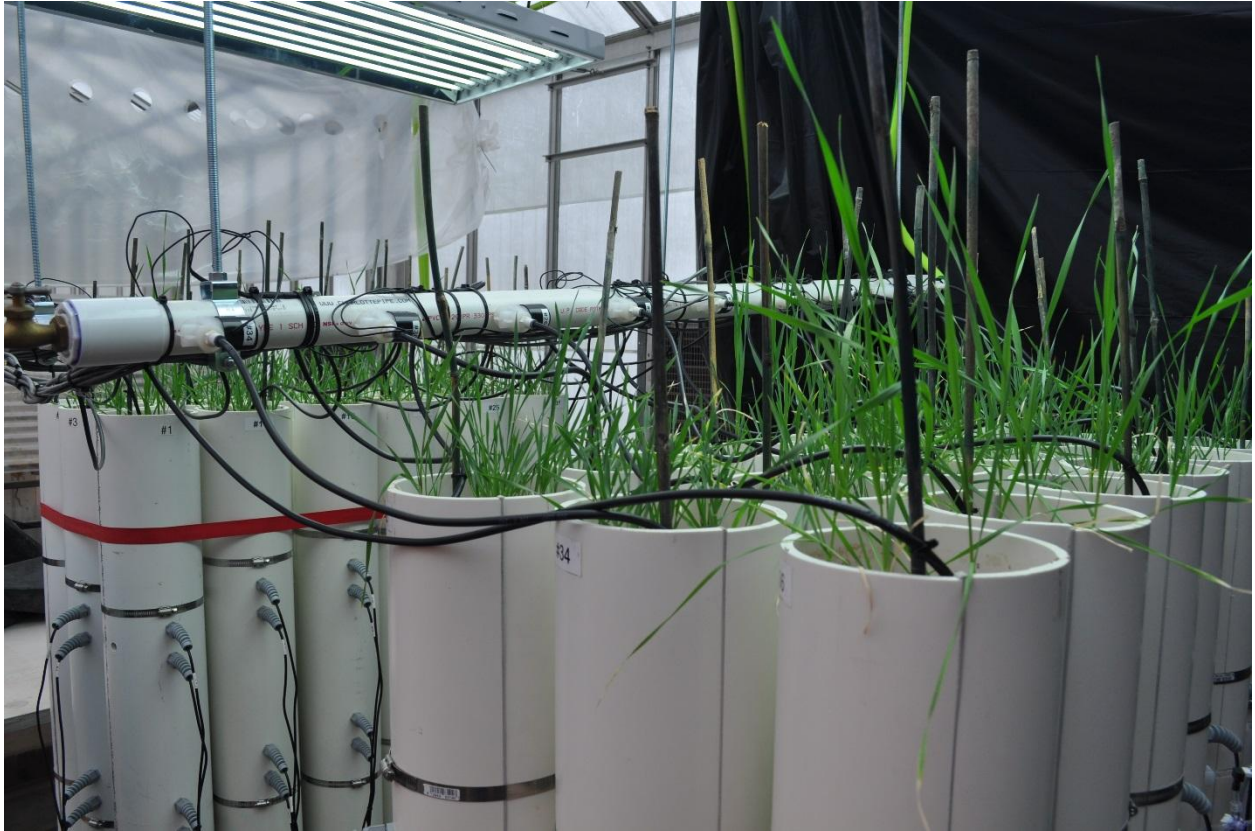


Figure 3-15 Irrigation Manifold with 12v Solenoid Valves Supplying Each Tube



Figure 3-16 Hole Securing ¼" Irrigation Tubing in each Growth Tube

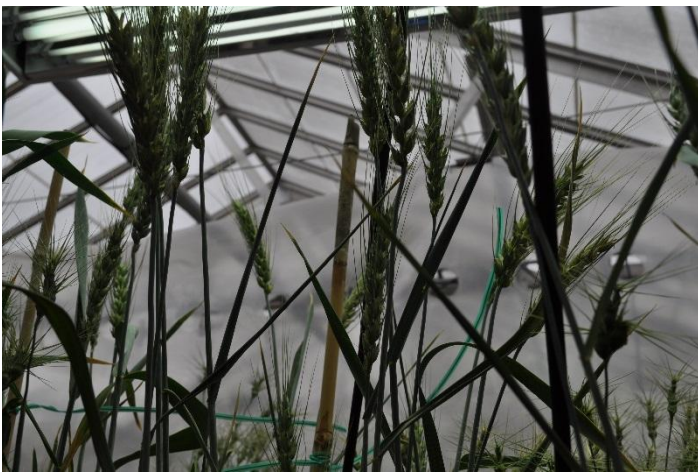


Figure 3-17 (clockwise) Lower Canopy of Well-watered Treatment; Leaf Stress of Lower Canopy of Drought Treatment; Comparison of Flag Leaf Stress Symptoms in Well-watered (right) versus Drought Stress (left); Wheat Flag Leaves Rolling during Drought Stress



Figure 3-18 Root System Evaluation at the Conclusion of Experiment 1. View of Tube with Intact Root System (top), Measurement of Rooting Depth (middle) and Sieve Separation of Root System and Growth Media (bottom)

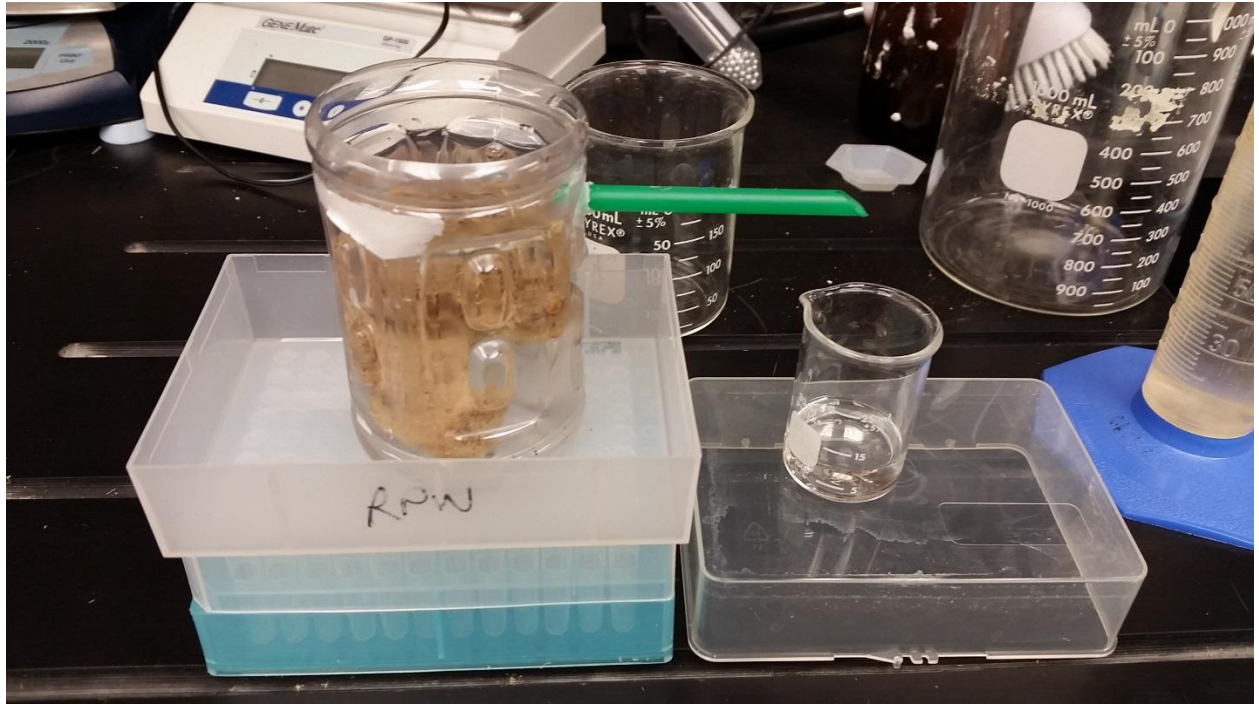


Figure 3-19 Root Volume Displacement Vessel

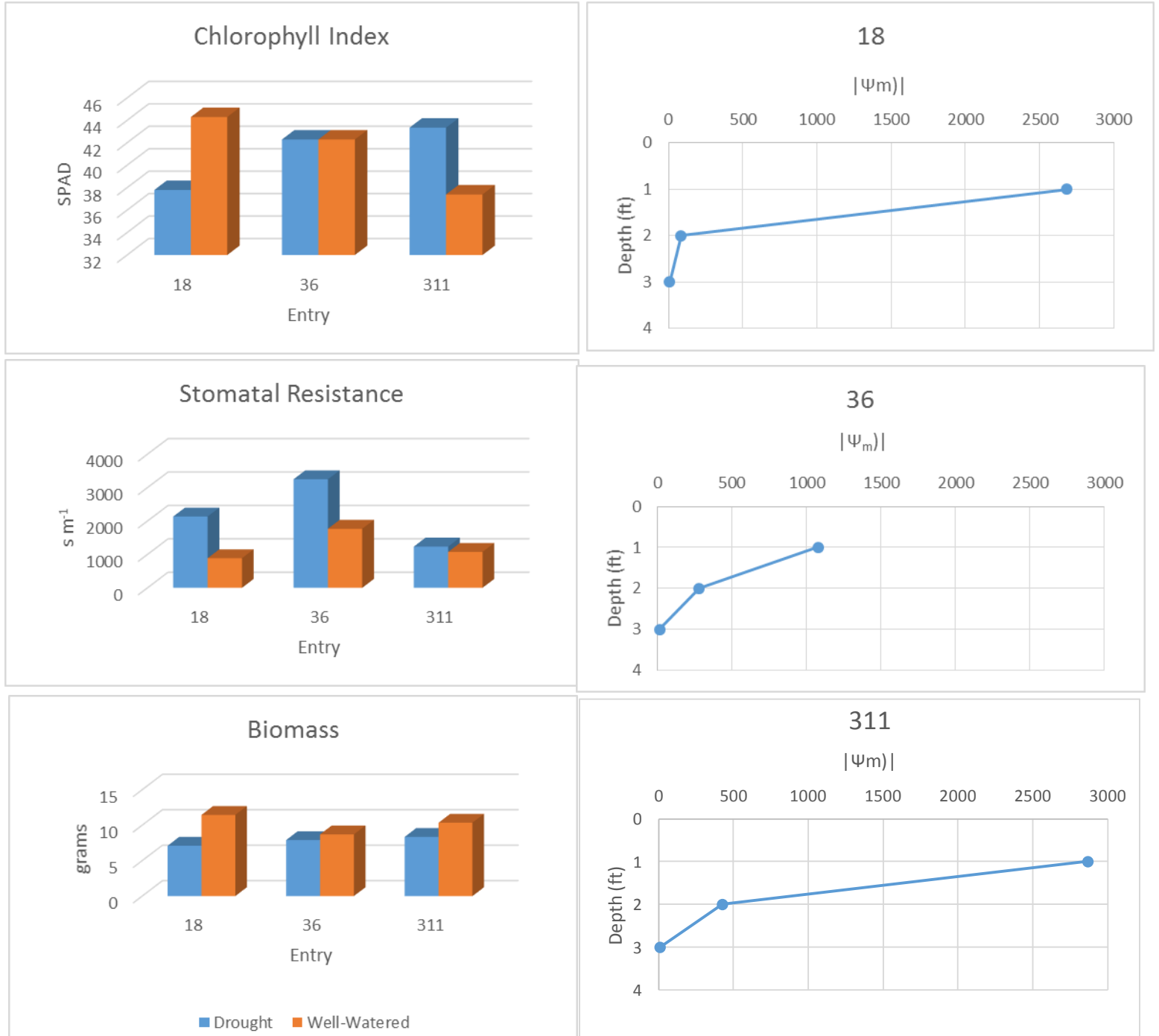


Figure 3-20 Final Measurement Comparison of Chlorophyll Index, Stomatal Resistance and Dry Biomass by Moisture Treatment for Entries 18, 36, and 311 (left) in Experiment 1. Matric Potential at Three Depths at Plant Senescence for Entries 18, 36, and 311 (right)

References

- Adams, C., A. Jacobson, and B. Bugbee. 2014. Ceramic Aggregate Sorption and Desorption Chemistry: Implications for Use as a Component of Soilless Media. *Journal of Plant Nutrition*, 37:1345-1357.
- Aghaee-Sarbarzeh M, M. Ferrahi, S. Singh, S. Harjit-Singh, B. Friebe, B.S. Gill, and HS. Dhaliwal. 2002. *PhI* induced transfer of leaf and stripe rust resistance genes from *Aegilops triuncialis* and *Ae. geniculata* to bread wheat. *Euphytica* 127:377–382
- Ali, M., C. R. Jensen, V. O. Mogensen, M. N. Andersen, and I. E. Henson. 1999. Root Signaling and Osmotic Adjustment during Intermittent Soil Drying Sustain Grain Yield of Field Grown Wheat. *Field Crops Research* 62:35-52.
- Babu, R.C., M.S. Pathan, A. Blum, and H.T. Nguyen. 1999. Comparison of Measurement Methods of Osmotic Adjustment in Rice Cultivars. *Crop Science*, 39:150-158.
- Barrs, H. D., and P. E. Weatherley. 1962. A Re-examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. *Australian Journal of Biological Sciences* 15: 413-428.
- Battenfield, S. D. A.R. Klatt, and W.R. Raun. 2013. Genetic Yield Potential Improvement of Semidwarf Winter Wheat in the Great Plains. *Crop Science* 53:946-955
- Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, FL.
- Blum, A., 1989. Osmotic Adjustment and Growth of Barley Genotypes under Drought Stress. *Crop Science* 29:230-233.
- Blum, A., 2005. Drought Resistance, Water-use Efficiency, and Yield Potential—Are they Compatible, Dissonant, or Mutually Exclusive? *Crop and Pasture Science* 56:1159-1168.

- Blum, A., 2009. Effective Use of Water (EUW) and not Water-Use Efficiency (WUE) is the Target of Crop Yield Improvement under Drought Stress. *Field Crops Research* 112:119-123.
- Blum, A. 2011. *Plant Breeding for Water-Limited Environments*. Springer, New York.
- Blum, A., 2015. Towards a conceptual ABA ideotype in plant breeding for water limited environments. *Functional Plant Biology* 42:502-513.
- Blum, A., H. Poiarkova, G. Golan, and J. Mayer. 1983. Effects of Translocation and Kernel Growth. *Field Crops Research* 6:51-58.
- Blum, A., J. Mayer, and G. Golan. 1988. The Effect of Grain Number per Ear (sink size) on Source Activity and its Water Relations in Wheat. *Journal of Experimental Botany* 39:106–114.
- Blum, A., L. Shpiler, G. Golan, and J. Mayer. 1989. Yield Stability and Canopy Temperature of Wheat Genotypes Under Drought Stress. *Field Crops Research* 22:289–296.
- Blum, A., C. Y. Sullivan, and H.T. Nguyen. 1997. The Effect of Plant Size on Wheat Response to Agents of Drought Stress. II. Water Deficit, Heat and ABA. *Functional Plant Biology* 24:43-48.
- Blum, A., J. Zhang, and H. T. Nguyen. 1999. Consistent Differences among Wheat Cultivars in Osmotic Adjustment and their Relationship to Plant Production. *Field Crops Research* 64:287-291.
- Chimenti, C.A., M. Marcantonio,, and A.J. Hall. 2006. Divergent Selection for Osmotic Adjustment Results in Improved Drought Tolerance in Maize (*Zea mays L.*) in both Early Growth and Flowering Phases. *Field Crops Research*, 95:305-315.

- Condon, Anthony G., R. A. Richards, G. J. Rebetzke, and G. D. Farquhar. 2004. Breeding for High Water-use Efficiency. *Journal of Experimental Botany* 55:2447-2460.
- Cornic, G. 2000. Drought Stress Inhibits Photosynthesis by Decreasing Stomatal Aperture—not by Affecting ATP Synthesis. *Trends in Plant Science*. 5:187-188.
- Farooq, S., 2001. Co-Existence of Salt and Drought Tolerance in Triticeae. *Hereditas* 135:205-210.
- Farooq, M., M. Hussain, A. Wahid, and K.H.M. Siddique. 2012. Drought Stress in Plants: An Overview. pp 1-33 In *Plant Responses to Drought Stress*. Springer Berlin Heidelberg.
- Fischer RA. 1981. Optimizing the Use of Water and Nitrogen through Breeding of Crops. *Plant and Soil* 58:249–278.
- Friebe, B., J. Jiang, W.J. Raupp, R.A. McIntosh, and B.S. Gill. 1996. Characterization of Wheat Alien Translocations Conferring Resistance to Diseases and Pests: Current Status. *Euphytica* 91:59-87.
- Gregersen, P. L., and P.B. Holm. 2007. Transcriptome Analysis of Senescence in the Flag Leaf of Wheat. *Plant Biotechnology Journal* 5: 192–206.
- Kirkham, M.B., W.R. Gardner, and G.C. Gerloff. 1969. Leaf Water Potential of Differentially Salinized Plants. *Plant Physiology* 44-1378-1382.
- Kirkham, M.B., 1983. Effect of Ethephon on the Water Status of a Drought-Resistant and a Drought Sensitive Cultivar of Winter Wheat. *Zeitschrift für Pflanzenphysiologie*, 112:103-112.
- Kirkham, M.B. *Principles of Soil and Plant Water Relations*. Elsevier, 2005.
- Kuraparthi, V., P. Chhuneja, H.S. Dhaliwal, S. Kaur, and B.S. Gill. 2007. Characterization and Mapping of *Aegilops geniculata* Introgressions with Novel Leaf Rust and Stripe Rust

- Resistance Genes Lr57 and Yr40 in Wheat. *Theoretical and Applied Genetics* 114:1379-1389.
- Mguis, K., A. Albouchi, A. Khadhri, M. Abassi, M. Yakoubi-Tej, A. Mahjoub, Z. Ouerghi, and N.B. Brahim. 2012. Adjustments in Leaf Water Relations of Wild Wheat Relative *Aegilops geniculata* Roth. and Wheat (*Triticum durum* Desf.) Plants Grown in a Salinity Gradient. *Australian Journal of Crop Science* 6:768-776.
- Mguis, K., A. Albouchi, M. Abassi, A. Khadhri, M. Ykoubi-Tej, A. Mahjoub, N.B. Brahim, and Z. Ouerghi. 2013. Responses of Leaf Growth and Gas Exchanges to Salt Stress during Reproductive Stage in Wild Wheat Relative *Aegilops geniculata* Roth. and Wheat (*Triticum durum* Desf.). *Acta physiologiae plantarum*, 35:1453-1461.
- Morgan, J. M., and A. G. Condon. 1986. Water Use, Grain Yield, and Osmoregulation in Wheat. *Functional Plant Biology* 13:523-532.
- Monneveux, P., M. Zaharieva, and D. Rekika. 2000. The Utilization of *Triticum* and *Aegilops* Species for the Improvement of Durum wheat. *Génétique et Amélioration des Plantes INRA*. 71-81.
- Munné-Bosch, S. and L. Alegre. 2004. Die and Let Live: Leaf Senescence Contributes to Plant Survival Under Drought Stress. *Functional Plant Biology* 31:203-216.
- Munns, R., and R. E. Sharp. 1993. Involvement of Abscisic Acid in Controlling Plant Growth in Soil of Low Water Potential. *Functional Plant Biology* 20:425-437.
- Musick, J. T., O.R. Jones, B.A. Stewart, and D.A. Dusek. 1994. Water-Yield Relationships for Irrigated and Dryland Wheat in the US Southern Plains. *Agronomy Journal* 86:980-986.

- O'Toole, J. C., and R.T. Cruz. 1980. Response of Leaf Water Potential, Stomatal Resistance, and Leaf Rolling to Water Stress. *Plant Physiology* 65:428-432.
- Pang, W., W. T. Crow, J. E. Luc, R. McSorley, R. M. Giblin-Davis, K. E. Kenworthy, and J. K. Kruse. 2011. Comparison of Water Displacement and WinRHIZO Software for Plant Root Parameter Assessment. *Plant Disease* 95:1308-1310.
- Pask, A.J.D., J. Pietragalla, D.M. Mullan, and M.P. Reynolds. 2012. *Physiological Breeding II: A Field Guide to Wheat Phenotyping*. CIMMYT.
- Plaut, Z., B.J. Butow, C.S. Blumenthal, and C.W. Wrigley. 2004. Transport of Dry Matter into Developing Wheat Kernels and its Contribution to Grain Yield under Post-Anthesis Water Deficit and Elevated Temperature. *Field Crops Research* 86: 185–198.
- Pradhan, G. P., P.V.V. Prasad, A.K. Fritz, M.B. Kirkham, and B.S. Gill. 2012. Effects of Drought and High Temperature Stress on Synthetic Hexaploid Wheat. *Functional Plant Biology* 39:190-198.
- Pradhan, G. P., Q. Xue, K.E. Jessup, J.C. Rudd, S. Liu, R.N. Devkota, and J.R. Mahan. 2014. Cooler Canopy Contributes to Higher Yield and Drought Tolerance in New Wheat Cultivars. *Crop Science*. 54:2275-2284.
- Rekika, D., MM. Nachit, J.L. Araus, and P. Monneveux. 1998a. Effects of Water Deficit on Photosynthetic Rate and Osmotic Adjustment in Tetraploid Wheats. *Photosynthetica*. 35: 129-138.
- Rekika, D., M. Zaharieva, P. Stankova, X. Xu, I. Souyris, and P. Monneveux. 1998b. Abiotic Stress Tolerance in *Aegilops* species. In: *Durum Wheat SEWANA Workshop*, Nachit, M.M., Baum, M., Porceddu, E., Monneveux, P. and Picard, E. (eds). ICARDA, Aleppo, Syria, pp. 113-128.

- Richards, R. A. 2006. Physiological Traits Used in the Breeding of New Cultivars for Water Scarce Environments. *Agricultural Water Management*. 80:197-211.
- Richards, R.A., G.J. Rebetzke, A.G. Condon, and A.F. Van Herwaarden. 2002. Breeding Opportunities for Increasing the Efficiency of Water Use and Crop Yield in Temperate Cereals. *Crop Science* 42:111-121
- SAS Institute, 2016. 9.4 foundation for 64-bit Microsoft Windows. SAS Inst., Cary, NC.
- Sreedhar, L., W.F. Wolkers, F.A. Hoekstra, and J.D. Bewley. 2002. In vivo Characterization of the Effects of Abscisic Acid and Drying Protocols Associated with the Acquisition of Desiccation Tolerance in Alfalfa (*Medicago sativa* L.) Somatic Embryos. *Annals of Botany* 89:391-400.
- Steinberg, S.L., G.J. Kluitenberg, S.B. Jones, N.E. Daidzic, L.N. Reddi, M. Xiao, M. Tuller, R.M. Newman, D. Or J.I.D. Alexander, J.I.D. 2005. Physical and Hydraulic Properties of Baked Ceramic Aggregates Used for Plant Growth Medium. *Journal of the American Society for Horticultural Science* 130:767-774.
- Sylvester-Bradley, R., R.K. Scott, and C.E. Wright. 1990. Physiology in the Production and Improvement of Cereals. *Home-Grown Cereals Authority Research Review* 18. HGCA, London.
- Tack, J., A. Barkley and L.L. Nalley. 2014. Heterogeneous Effects of Warming and Drought on Selected Wheat Variety Yields. *Climatic Change* 125:489-500.
- Turner, N. C. 1979. Drought Resistance and Adaptation to Water Deficits in Crop Plants. In: *Stress Physiology in Crop Plants*. pp. 343–372. H. Mussell and C.R. Staples, Eds., John Wiley & Sons, New York

- Wasson, A. P., R.A. Richards, R. Chatrath, S.C. Misra, S.V. Sai Prasad, G.J. Rebetzke, J.A. Kirkegaard, J. Christopher, and M. Watt. 2012. Traits and Selection Strategies to Improve Root Systems and Water Uptake in Water Limited Wheat Crops. *Journal of Experimental Botany* 63: 3485-3498.
- Watson, Steve. 2015. *Wheat Varieties for Kansas and the Great Plains, 2015*. Lone Tree Publishing, Topeka, Kansas.
- Xue Q., Z. Zhu, J.T. Musick, B.A. Stewart, and D.A. Dusek. 2003. Root Growth and Water Uptake in Winter Wheat Under Deficit Irrigation. *Plant and Soil* 257:151-161.
- Xue Q., J.C. Rudd, S. Liu, K.E. Jessup, R.N. Devkota, and J.R. Mahan. 2014. Yield Determination and Water Use Efficiency of Wheat Under Water-Limited Conditions in the U.S. Southern High Plains. *Crop Science*. 54: 34-47.
- Yang, J., J. Zhang, Z. Wang, Q. Zhu and L. Liu. 2001. Water Deficit Induced Senescence and its Relationship to the Remobilization of Pre-stored Carbon in Wheat during Grain Filling. *Agronomy Journal*. 93: 196–206.
- Yang, J., J. Zhang Z. Wang, Q. Zhu, and L. Liu. 2003. Involvement of Abscisic Acid and Cytokinins in the Senescence and Remobilization of Carbon Reserves in Wheat Subjected to Water Stress during Grain Filling. *Plant Cell and Environment* 26: 1621-1631.
- Zaharieva, M., E. Gaulin, M. Havaux, E. Acevedo, and P. Monneveux. 2001. Drought and Heat Responses in the Wild Wheat Relative *Aegilops geniculata* Roth: Potential Interest for Wheat Improvement. *Crop Science* 41:1321–1329.
- Zhang, Jingxian, H. T. Nguyen, and A. Blum. 1999. Genetic Analysis of Osmotic Adjustment in Crop Plants. *Journal of Experimental Botany* 50:291-302.

Appendix A - Advanced Drought Screening System Parts List

Tubes

Solid core SCH-40 6" PVC pipe, Environmental Manufacturing, Manhattan, KS
PCG 3/8-R Cable Gland, Mencom Corporation, Eagle Sensors and Controls, Lenexa, KS
6" PVC Coupling-NDS Drainage Products, Model Number: M6P05 | Menards® SKU: 6890418
6" Round Drain Grate- NDS Drainage Products, Model M50 Menards® SKU: 6899325
6-7" hose clamp- Breeze, Model Number: 63104MEB | Menards® SKU: 6790649
Closed-Cell Vinyl Foam Tape Weatherstrip-WJ Dennis Model Number:
(1/2") 204 | Menards® SKU: 567205
(1/4") Model Number: 201 | Menards® SKU: 5672035
Size 60 Type 304 stainless steel mesh, TWP Inc. Berkeley, CA Part # 060X060S0065W36T

Irrigation System

Flexzilla 50 foot 5/8" garden hose, Model Number: HFZG550YW Menards® SKU: 2741205
13 gallon Nalgene polypropylene carboy with spigot, United States Plastic Corp, Item #73090
Model 8005-233-236 1.4 GPM pump, Shurflo, Cypress CA
Peters Professional 5-11-26 Hydroponic special fertilizer, Hummert International, Earth City,
MO Catalog number 07-5570-1
Calcium nitrate tetrahydrate, Educational Grade, Fisher Scientific, Waltham, MA. Catalog
number S25226A
Schedule 40 Solid Core Plain End Pipe-1-1/4"x5' Model
Number: PVC071001000HCMenards® SKU: 6898533
1-1/4"x1" Bushing PVC Schedule 40 Model Number: F02020D | Menards® SKU: 6897107
1-1/4" Coupling PVC Schedule 40 Model Number: F00040D | Menards® SKU: 6891860
3/4" male Hose x Male hose Model Number: 0122229 | Menards® SKU: 6801973
1-1/4"x 3/4" Bushing PVC Schedule 40 Model Number: F01270D Menards® SKU: 6897136
3/4" Hose Bibb Male Model Number: 0960121 Menards® SKU: 6851686
1/8" 12v DC plastic solenoid valve, Electric Solenoid Valves (Holbrook, NY) Part# RSC-212V
1/4 IN OD (1/8 ID) x 1/4 IN MIP Plastic Quick-Connect Male Adapter Model
Number: 17103005 Menards® SKU: 6806152
1/8" MIP Lead Free Brass Pipe Hex Nipple Model Number: 0123926 Menards® SKU: 6805982

Sensors

MPS-6 Matric Potential Sensor, Decagon Devices, Pullman, WA
EC-5 Volumetric Water Content Sensor, Decagon Devices, Pullman, WA
200 mesh silica flour, Soil Moisture Equipment, Santa Barbara, CA Catalog# 0930W050
Belmont, CA Part # 2218530
Flow cell tensiometer 16cm long to porous cup (.6cm OD cup), with 3/8" NPT compression
fitting, 3-way valve, pressure transducer (5psi), and 5 meter 4 conductor wire and plug,
(Soil Measurement Systems, Huntington Beach, CA) Item # CI-029B
Deutsch Rectangle 12 wire EC-5 plug for optimal treatment, pin side. Part# DTM04-12PA-L012
(LADD, Kettering, OH)
Deutsch Rectangle 12 wire irrigation plug for optimal treatment, pin side. Part# DTM04-12PB
L012 (LADD, Kettering, OH)

Deutsch Rectangle 12 wire EC-5 plug for optimal treatment, socket side. Part# DTM06-12SA (LADD, Kettering, OH)
Deutsch Rectangle 12 wire irrigation plug for optimal treatment, socket. Part# DTM06 12SB(LADD, Kettering, OH)
Deutsch Pin Wedge Lock. Part# WM-12P. (LADD, Kettering, OH)
Deutsch Socket Wedge Lock. Part# WM-12S (LADD, Kettering, OH)
Deutsch Round 21 wire Drought Tube Plug, Socket side. Part# HDP24-18-21SN (LADD, Kettering, OH)
Deutsch Round 21 wire Drought Tube Plug, Pin side. Part# HDP26-18-21PN (LADD, Kettering, OH)
Deutsch Solid size 20AWG nickel pins. Part# 0460-202-20141(LADD, Kettering, OH)
Deutsch Solid size 20AWG nickel sockets. Part# 0462-201-20141(LADD, Kettering, OH)
Deutsch Pin and socket connections made with Deutsch HDT-48-00 hand tool (LADD, Kettering, OH)

Infrastructure

22 AWG, 4 Conductor, Communication Cable, Unshielded, Wire and Cable to Go, Highland Park, IL Catalog# COM-C4063A-500
Profile Greens Grade, (Profile Products LLC, Buffalo Grove, IL)
Osmocote Plus 15-9-12 3-4 month, Hummert International, Earth City, MO
Sunblaze 4'x8 lamp T5 HO Grow Lights with Spectralux 6500K lamps, Hummert, Earth City, MO

Data Collection System

Single Output DIN Rail Power Supply 12 Volts 10 Amps 120 Watts, Jameco Electronics, AM1632B multiplexer, Campbell Scientific, Logan, UT
CR1000 datalogger, Campbell Scientific, Logan, UT
PS150 Power Supply, Campbell Scientific, Logan, UT
118 cfm 12v DC Brushless fan, Jameco Electronics, Belmont, CA Part # 1952791
5" DIN rail terminal kit, Part 25458, Campbell Scientific, Logan, UT

Instrumentation

Decagon SC-1 Leaf Porometer, Decagon Devices, Pullman WA
Konica-Minolta SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Aurora, IL
Model 1000 Pressure Chamber, PMS Instrument Company, Albany, OR

**Appendix B - CR1000 (1) Programming (EC-5; MPS-6; and
Tensiometers 1-32)**

Dim LCount
Dim LCount_2
Dim LCount_3
Dim LCount_4
Dim LCount_5
Dim LCount_6
Dim LCount_7
Dim LCount_8
Dim LCount_9
Dim LCount_10
Dim LCount_11
Dim LCount_12
Dim LCount_13
Dim LCount_14
Dim LCount_15
Dim LCount_16
Dim LCount_17
Dim LCount_18
Dim LCount_19
Dim LCount_20
Dim LCount_21
Dim LCount_22
Dim LCount_23
Dim LCount_24
Dim LCount_25
Dim LCount_26
Dim LCount_27
Dim LCount_28
Dim LCount_29
Dim LCount_30
Dim LCount_31
Dim LCount_32
Dim LCount_33
Dim LCount_34
Dim LCount_35
Dim LCount_36
Dim LCount_37
Dim LCount_38
Dim LCount_39
Dim LCount_40

Dim LCount_41
Dim LCount_42
Dim LCount_43
Dim LCount_44
Dim LCount_45
Dim LCount_46
Dim LCount_47
Dim LCount_48
Dim LCount_49
Dim LCount_50
Dim LCount_51
Dim LCount_52
Dim LCount_53
Dim LCount_54
Dim LCount_55
Dim LCount_56
Dim LCount_57
Dim LCount_58
Dim LCount_59
Dim LCount_60
Dim LCount_61
Dim LCount_62
Dim LCount_63
Dim LCount_64
Dim LCount_65
Dim LCount_66
Dim LCount_67
Dim LCount_68
Dim LCount_69
Dim LCount_70
Dim LCount_71
Dim LCount_72
Dim LCount_73
Dim LCount_74
Dim LCount_75
Dim LCount_76
Dim LCount_77
Dim LCount_78
Dim LCount_79
Dim LCount_80
Dim LCount_81
Dim LCount_82
Dim LCount_83
Dim LCount_84
Dim LCount_85
Dim LCount_86

Dim LCount_87
Dim LCount_88
Dim LCount_89
Dim LCount_90
Dim LCount_91
Dim LCount_92
Dim LCount_93
Dim LCount_94
Dim LCount_95
Dim LCount_96
Dim LCount_97
Dim LCount_98
Dim LCount_99
Dim LCount_100
Dim LCount_101
Dim LCount_102
Dim LCount_103
Dim LCount_104
Dim LCount_105
Dim LCount_106
Dim LCount_107
Dim LCount_108
Dim LCount_109
Dim LCount_110
Dim LCount_111
Dim LCount_112
Dim LCount_113
Dim LCount_114
Dim LCount_115
Dim LCount_116
Dim LCount_117
Dim LCount_118
Dim LCount_119
Dim LCount_120
Dim LCount_121
Dim LCount_122
Dim LCount_123
Dim LCount_124
Dim LCount_125
Dim LCount_126
Dim LCount_127
Dim LCount_128
Dim LCount_129
Dim LCount_130
Dim LCount_131
Dim LCount_132

Dim LCount_133
Dim LCount_134
Dim LCount_135
Dim LCount_136
Dim LCount_137
Dim LCount_138
Dim LCount_139
Dim LCount_140
Dim LCount_141
Dim LCount_142
Dim LCount_143
Dim LCount_144
Public BattV
Public PTemp_C
Public VW_1 **'these variables are the millivolt output which have not been converted
to VWC yet**
Public VW_2
Public VW_3
Public VW_4
Public VW_5
Public VW_6
Public VW_7
Public VW_8
Public VW_9
Public VW_10
Public VW_11
Public VW_12
Public VW_13
Public VW_14
Public VW_15
Public VW_16
Public VW_17
Public VW_18
Public VW_19
Public VW_20
Public VW_21
Public VW_22
Public VW_23
Public VW_24
Public VW_25
Public VW_26
Public VW_27
Public VW_28
Public VW_29
Public VW_30
Public VW_31

Public VW_32
Public VW_33
Public VW_34
Public VW_35
Public VW_36
Public VW_37
Public VW_38
Public VW_39
Public VW_40
Public VW_41
Public VW_42
Public VW_43
Public VW_44
Public VW_45
Public VW_46
Public VW_47
Public VW_48
Public VW_49
Public VW_50
Public VW_51
Public VW_52
Public VW_53
Public VW_54
Public VW_55
Public VW_56
Public VW_57
Public VW_58
Public VW_59
Public VW_60
Public VW_61
Public VW_62
Public VW_63
Public VW_64
Public VW_65
Public VW_66
Public VW_67
Public VW_68
Public VW_69
Public VW_70
Public VW_71
Public VW_72
Public VW_73
Public VW_74
Public VW_75
Public VW_76
Public VW_77

Public VW_78
Public VW_79
Public VW_80
Public VW_81
Public VW_82
Public VW_83
Public VW_84
Public VW_85
Public VW_86
Public VW_87
Public VW_88
Public VW_89
Public VW_90
Public VW_91
Public VW_92
Public VW_93
Public VW_94
Public VW_95
Public VW_96
Public VW_97
Public VW_98
Public VW_99
Public VW_100
Public VW_101
Public VW_102
Public VW_103
Public VW_104
Public VW_105
Public VW_106
Public VW_107
Public VW_108
Public VW_109
Public VW_110
Public VW_111
Public VW_112
Public VW_113
Public VW_114
Public VW_115
Public VW_116
Public VW_117
Public VW_118
Public VW_119
Public VW_120
Public VW_121
Public VW_122
Public VW_123

Public VW_124
Public VW_125
Public VW_126
Public VW_127
Public VW_128
Public VW_129
Public VW_130
Public VW_131
Public VW_132
Public VW_133
Public VW_134
Public VW_135
Public VW_136
Public VW_137
Public VW_138
Public VW_139
Public VW_140
Public VW_141
Public VW_142
Public VW_143
Public VW_144
Public VWC_1
Public VWC_2
Public VWC_3
Public VWC_4
Public VWC_5
Public VWC_6
Public VWC_7
Public VWC_8
Public VWC_9
Public VWC_10
Public VWC_11
Public VWC_12
Public VWC_13
Public VWC_14
Public VWC_15
Public VWC_16
Public VWC_17
Public VWC_18
Public VWC_19
Public VWC_20
Public VWC_21
Public VWC_22
Public VWC_23
Public VWC_24
Public VWC_25

Public VWC_26
Public VWC_27
Public VWC_28
Public VWC_29
Public VWC_30
Public VWC_31
Public VWC_32
Public VWC_33
Public VWC_34
Public VWC_35
Public VWC_36
Public VWC_37
Public VWC_38
Public VWC_39
Public VWC_40
Public VWC_41
Public VWC_42
Public VWC_43
Public VWC_44
Public VWC_45
Public VWC_46
Public VWC_47
Public VWC_48
Public VWC_49
Public VWC_50
Public VWC_51
Public VWC_52
Public VWC_53
Public VWC_54
Public VWC_55
Public VWC_56
Public VWC_57
Public VWC_58
Public VWC_59
Public VWC_60
Public VWC_61
Public VWC_62
Public VWC_63
Public VWC_64
Public VWC_65
Public VWC_66
Public VWC_67
Public VWC_68
Public VWC_69
Public VWC_70
Public VWC_71

Public VWC_72
Public VWC_73
Public VWC_74
Public VWC_75
Public VWC_76
Public VWC_77
Public VWC_78
Public VWC_79
Public VWC_80
Public VWC_81
Public VWC_82
Public VWC_83
Public VWC_84
Public VWC_85
Public VWC_86
Public VWC_87
Public VWC_88
Public VWC_89
Public VWC_90
Public VWC_91
Public VWC_92
Public VWC_93
Public VWC_94
Public VWC_95
Public VWC_96
Public VWC_97
Public VWC_98
Public VWC_99
Public VWC_100
Public VWC_101
Public VWC_102
Public VWC_103
Public VWC_104
Public VWC_105
Public VWC_106
Public VWC_107
Public VWC_108
Public VWC_109
Public VWC_110
Public VWC_111
Public VWC_112
Public VWC_113
Public VWC_114
Public VWC_115
Public VWC_116
Public VWC_117

Public VWC_118
Public VWC_119
Public VWC_120
Public VWC_121
Public VWC_122
Public VWC_123
Public VWC_124
Public VWC_125
Public VWC_126
Public VWC_127
Public VWC_128
Public VWC_129
Public VWC_130
Public VWC_131
Public VWC_132
Public VWC_133
Public VWC_134
Public VWC_135
Public VWC_136
Public VWC_137
Public VWC_138
Public VWC_139
Public VWC_140
Public VWC_141
Public VWC_142
Public VWC_143
Public VWC_144

'Tensiometer 1 and 2 Full Bridge measurements

Public LCount145

Public T_1(16) **'for simplicity, the tensiometer measurements are made as T (1-16)**
and T_2 (17-32)

Public LCount146

Public T_2(16)

Public Mult(16)={1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1}

Public Offs(16)={0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0}

Public Mult_2(16)={1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1}

Public Offs_2(16)={0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0}

'Declare Tensiometer results with calibration equation

Public T_kPa_1

Public T_kPa_2

Public T_kPa_3

Public T_kPa_4

Public T_kPa_5

Public T_kPa_6

Public T_kPa_7

Public T_kPa_8

Public T_kPa_9
Public T_kPa_10
Public T_kPa_11
Public T_kPa_12
Public T_kPa_13
Public T_kPa_14
Public T_kPa_15
Public T_kPa_16
Public T_kPa_17
Public T_kPa_18
Public T_kPa_19
Public T_kPa_20
Public T_kPa_21
Public T_kPa_22
Public T_kPa_23
Public T_kPa_24
Public T_kPa_25
Public T_kPa_26
Public T_kPa_27
Public T_kPa_28
Public T_kPa_29
Public T_kPa_30
Public T_kPa_31
Public T_kPa_32

'Each of 54 SDI-12 measurements with the MPS-6 sensors is declared. The (2) after the sensor number means that there are two values returned, kPa and DegC

Public SDI12_1(2)
Public SDI12_2(2)
Public SDI12_3(2)
Public SDI12_4(2)
Public SDI12_5(2)
Public SDI12_6(2)
Public SDI12_7(2)
Public SDI12_8(2)
Public SDI12_9(2)
Public SDI12_10(2)
Public SDI12_11(2)
Public SDI12_12(2)
Public SDI12_13(2)
Public SDI12_14(2)
Public SDI12_15(2)
Public SDI12_16(2)
Public SDI12_17(2)
Public SDI12_18(2)
Public SDI12_19(2)
Public SDI12_20(2)

Public SDI12_21(2)
Public SDI12_22(2)
Public SDI12_23(2)
Public SDI12_24(2)
Public SDI12_25(2)
Public SDI12_26(2)
Public SDI12_27(2)
Public SDI12_28(2)
Public SDI12_29(2)
Public SDI12_30(2)
Public SDI12_31(2)
Public SDI12_32(2)
Public SDI12_33(2)
Public SDI12_34(2)
Public SDI12_35(2)
Public SDI12_36(2)
Public SDI12_37(2)
Public SDI12_38(2)
Public SDI12_39(2)
Public SDI12_40(2)
Public SDI12_41(2)
Public SDI12_42(2)
Public SDI12_43(2)
Public SDI12_44(2)
Public SDI12_45(2)
Public SDI12_46(2)
Public SDI12_47(2)
Public SDI12_48(2)
Public SDI12_49(2)
Public SDI12_50(2)
Public SDI12_51(2)
Public SDI12_52(2)
Public SDI12_53(2)
Public SDI12_54(2)
Dim LCount148

'Name the two resultant values for the MPS-6 sensors as kPa (first result) and DegC (second result)-these must be in this order

Alias SDI12_1(1)=M1_kPa
Alias SDI12_1(2)=M1_DegC
Alias SDI12_2(1)=M2_kPa
Alias SDI12_2(2)=M2_DegC
Alias SDI12_3(1)=M3_kPa
Alias SDI12_3(2)=M3_DegC
Alias SDI12_4(1)=M4_kPa
Alias SDI12_4(2)=M4_DegC
Alias SDI12_5(1)=M5_kPa

Alias SDI12_5(2)=M5_DegC
Alias SDI12_6(1)=M6_kPa
Alias SDI12_6(2)=M6_DegC
Alias SDI12_7(1)=M7_kPa
Alias SDI12_7(2)=M7_DegC
Alias SDI12_8(1)=M8_kPa
Alias SDI12_8(2)=M8_DegC
Alias SDI12_9(1)=M9_kPa
Alias SDI12_9(2)=M9_DegC
Alias SDI12_10(1)=M10_kPa
Alias SDI12_10(2)=M10_DegC
Alias SDI12_11(1)=M11_kPa
Alias SDI12_11(2)=M11_DegC
Alias SDI12_12(1)=M12_kPa
Alias SDI12_12(2)=M12_DegC
Alias SDI12_13(1)=M13_kPa
Alias SDI12_13(2)=M13_DegC
Alias SDI12_14(1)=M14_kPa
Alias SDI12_14(2)=M14_DegC
Alias SDI12_15(1)=M15_kPa
Alias SDI12_15(2)=M15_DegC
Alias SDI12_16(1)=M16_kPa
Alias SDI12_16(2)=M16_DegC
Alias SDI12_17(1)=M17_kPa
Alias SDI12_17(2)=M17_DegC
Alias SDI12_18(1)=M18_kPa
Alias SDI12_18(2)=M18_DegC
Alias SDI12_19(1)=M19_kPa
Alias SDI12_19(2)=M19_DegC
Alias SDI12_20(1)=M20_kPa
Alias SDI12_20(2)=M20_DegC
Alias SDI12_21(1)=M21_kPa
Alias SDI12_21(2)=M21_DegC
Alias SDI12_22(1)=M22_kPa
Alias SDI12_22(2)=M22_DegC
Alias SDI12_23(1)=M23_kPa
Alias SDI12_23(2)=M23_DegC
Alias SDI12_24(1)=M24_kPa
Alias SDI12_24(2)=M24_DegC
Alias SDI12_25(1)=M25_kPa
Alias SDI12_25(2)=M25_DegC
Alias SDI12_26(1)=M26_kPa
Alias SDI12_26(2)=M26_DegC
Alias SDI12_27(1)=M27_kPa
Alias SDI12_27(2)=M27_DegC
Alias SDI12_28(1)=M28_kPa

Alias SDI12_28(2)=M28_DegC
Alias SDI12_29(1)=M29_kPa
Alias SDI12_29(2)=M29_DegC
Alias SDI12_30(1)=M30_kPa
Alias SDI12_30(2)=M30_DegC
Alias SDI12_31(1)=M31_kPa
Alias SDI12_31(2)=M31_DegC
Alias SDI12_32(1)=M32_kPa
Alias SDI12_32(2)=M32_DegC
Alias SDI12_33(1)=M33_kPa
Alias SDI12_33(2)=M33_DegC
Alias SDI12_34(1)=M34_kPa
Alias SDI12_34(2)=M34_DegC
Alias SDI12_35(1)=M35_kPa
Alias SDI12_35(2)=M35_DegC
Alias SDI12_36(1)=M36_kPa
Alias SDI12_36(2)=M36_DegC
Alias SDI12_37(1)=M37_kPa
Alias SDI12_37(2)=M37_DegC
Alias SDI12_38(1)=M38_kPa
Alias SDI12_38(2)=M38_DegC
Alias SDI12_39(1)=M39_kPa
Alias SDI12_39(2)=M39_DegC
Alias SDI12_40(1)=M40_kPa
Alias SDI12_40(2)=M40_DegC
Alias SDI12_41(1)=M41_kPa
Alias SDI12_41(2)=M41_DegC
Alias SDI12_42(1)=M42_kPa
Alias SDI12_42(2)=M42_DegC
Alias SDI12_43(1)=M43_kPa
Alias SDI12_43(2)=M43_DegC
Alias SDI12_44(1)=M44_kPa
Alias SDI12_44(2)=M44_DegC
Alias SDI12_45(1)=M45_kPa
Alias SDI12_45(2)=M45_DegC
Alias SDI12_46(1)=M46_kPa
Alias SDI12_46(2)=M46_DegC
Alias SDI12_47(1)=M47_kPa
Alias SDI12_47(2)=M47_DegC
Alias SDI12_48(1)=M48_kPa
Alias SDI12_48(2)=M48_DegC
Alias SDI12_49(1)=M49_kPa
Alias SDI12_49(2)=M49_DegC
Alias SDI12_50(1)=M50_kPa
Alias SDI12_50(2)=M50_DegC
Alias SDI12_51(1)=M51_kPa

Alias SDI12_51(2)=M51_DegC
Alias SDI12_52(1)=M52_kPa
Alias SDI12_52(2)=M52_DegC
Alias SDI12_53(1)=M53_kPa
Alias SDI12_53(2)=M53_DegC
Alias SDI12_54(1)=M54_kPa
Alias SDI12_54(2)=M54_DegC

Units BattV=Volts

Units PTemp_C=Deg C

Units T_1=mV/V **'unit value declaration for the two tensiometer multiplexers- mv/V is converted to kPa by the calibration equation after the measurement section**

Units T_2=mV/V

DataTable(Tens,True,-1)

DataInterval(0,180,min,10) **'Second number shows interval of storing data to the DataTable named in the line above**

Sample(1,T_kPa_1,FP2) **'first report the kPa output after the calibration equation- then record the raw mv/V output below**

Sample(1,T_kPa_2,FP2)

Sample(1,T_kPa_3,FP2)

Sample(1,T_kPa_4,FP2)

Sample(1,T_kPa_5,FP2)

Sample(1,T_kPa_6,FP2)

Sample(1,T_kPa_7,FP2)

Sample(1,T_kPa_8,FP2)

Sample(1,T_kPa_9,FP2)

Sample(1,T_kPa_10,FP2)

Sample(1,T_kPa_11,FP2)

Sample(1,T_kPa_12,FP2)

Sample(1,T_kPa_13,FP2)

Sample(1,T_kPa_14,FP2)

Sample(1,T_kPa_15,FP2)

Sample(1,T_kPa_16,FP2)

Sample(1,T_kPa_17,FP2)

Sample(1,T_kPa_18,FP2)

Sample(1,T_kPa_19,FP2)

Sample(1,T_kPa_20,FP2)

Sample(1,T_kPa_21,FP2)

Sample(1,T_kPa_22,FP2)

Sample(1,T_kPa_23,FP2)

Sample(1,T_kPa_24,FP2)

Sample(1,T_kPa_25,FP2)

Sample(1,T_kPa_26,FP2)

Sample(1,T_kPa_27,FP2)

Sample(1,T_kPa_28,FP2)

Sample(1,T_kPa_29,FP2)

Sample(1,T_kPa_30,FP2)

Sample(1,T_kPa_31,FP2)
 Sample(1,T_kPa_32,FP2)
 Sample(1,T_1(1),FP2)
 Sample(1,T_1(2),FP2)
 Sample(1,T_1(3),FP2)
 Sample(1,T_1(4),FP2)
 Sample(1,T_1(5),FP2)
 Sample(1,T_1(6),FP2)
 Sample(1,T_1(7),FP2)
 Sample(1,T_1(8),FP2)
 Sample(1,T_1(9),FP2)
 Sample(1,T_1(10),FP2)
 Sample(1,T_1(11),FP2)
 Sample(1,T_1(12),FP2)
 Sample(1,T_1(13),FP2)
 Sample(1,T_1(14),FP2)
 Sample(1,T_1(15),FP2)
 Sample(1,T_1(16),FP2)
 Sample(1,T_2(1),FP2)
 Sample(1,T_2(2),FP2)
 Sample(1,T_2(3),FP2)
 Sample(1,T_2(4),FP2)
 Sample(1,T_2(5),FP2)
 Sample(1,T_2(6),FP2)
 Sample(1,T_2(7),FP2)
 Sample(1,T_2(8),FP2)
 Sample(1,T_2(9),FP2)
 Sample(1,T_2(10),FP2)
 Sample(1,T_2(11),FP2)
 Sample(1,T_2(12),FP2)
 Sample(1,T_2(13),FP2)
 Sample(1,T_2(14),FP2)
 Sample(1,T_2(15),FP2)
 Sample(1,T_2(16),FP2)
 End Table
 DataTable(VWC,True,-1)
 DataInterval(0,180,min,10)
 Sample (1,VWC_1,FP2)
'Converted VWC is stored first (1-144), then VW as raw value (mv) for future use
 Sample(1,VWC_2,FP2)
 Sample(1,VWC_3,FP2)
 Sample(1,VWC_4,FP2)
 Sample(1,VWC_5,FP2)
 Sample(1,VWC_6,FP2)
 Sample(1,VWC_7,FP2)
 Sample(1,VWC_8,FP2)

Sample(1,VWC_9,FP2)
Sample(1,VWC_10,FP2)
Sample(1,VWC_11,FP2)
Sample(1,VWC_12,FP2)
Sample(1,VWC_13,FP2)
Sample(1,VWC_14,FP2)
Sample(1,VWC_15,FP2)
Sample(1,VWC_16,FP2)
Sample(1,VWC_17,FP2)
Sample(1,VWC_18,FP2)
Sample(1,VWC_19,FP2)
Sample(1,VWC_20,FP2)
Sample(1,VWC_21,FP2)
Sample(1,VWC_22,FP2)
Sample(1,VWC_23,FP2)
Sample(1,VWC_24,FP2)
Sample(1,VWC_25,FP2)
Sample(1,VWC_26,FP2)
Sample(1,VWC_27,FP2)
Sample(1,VWC_28,FP2)
Sample(1,VWC_29,FP2)
Sample(1,VWC_30,FP2)
Sample(1,VWC_31,FP2)
Sample(1,VWC_32,FP2)
Sample(1,VWC_33,FP2)
Sample(1,VWC_34,FP2)
Sample(1,VWC_35,FP2)
Sample(1,VWC_36,FP2)
Sample(1,VWC_37,FP2)
Sample(1,VWC_38,FP2)
Sample(1,VWC_39,FP2)
Sample(1,VWC_40,FP2)
Sample(1,VWC_41,FP2)
Sample(1,VWC_42,FP2)
Sample(1,VWC_43,FP2)
Sample(1,VWC_44,FP2)
Sample(1,VWC_45,FP2)
Sample(1,VWC_46,FP2)
Sample(1,VWC_47,FP2)
Sample(1,VWC_48,FP2)
Sample(1,VWC_49,FP2)
Sample(1,VWC_50,FP2)
Sample(1,VWC_51,FP2)
Sample(1,VWC_52,FP2)
Sample(1,VWC_53,FP2)
Sample(1,VWC_54,FP2)

Sample(1,VWC_55,FP2)
Sample(1,VWC_56,FP2)
Sample(1,VWC_57,FP2)
Sample(1,VWC_58,FP2)
Sample(1,VWC_59,FP2)
Sample(1,VWC_60,FP2)
Sample(1,VWC_61,FP2)
Sample(1,VWC_62,FP2)
Sample(1,VWC_63,FP2)
Sample(1,VWC_64,FP2)
Sample(1,VWC_65,FP2)
Sample(1,VWC_66,FP2)
Sample(1,VWC_67,FP2)
Sample(1,VWC_68,FP2)
Sample(1,VWC_69,FP2)
Sample(1,VWC_70,FP2)
Sample(1,VWC_71,FP2)
Sample(1,VWC_72,FP2)
Sample(1,VWC_73,FP2)
Sample(1,VWC_74,FP2)
Sample(1,VWC_75,FP2)
Sample(1,VWC_76,FP2)
Sample(1,VWC_77,FP2)
Sample(1,VWC_78,FP2)
Sample(1,VWC_79,FP2)
Sample(1,VWC_80,FP2)
Sample(1,VWC_81,FP2)
Sample(1,VWC_82,FP2)
Sample(1,VWC_83,FP2)
Sample(1,VWC_84,FP2)
Sample(1,VWC_85,FP2)
Sample(1,VWC_86,FP2)
Sample(1,VWC_87,FP2)
Sample(1,VWC_88,FP2)
Sample(1,VWC_89,FP2)
Sample(1,VWC_90,FP2)
Sample(1,VWC_91,FP2)
Sample(1,VWC_92,FP2)
Sample(1,VWC_93,FP2)
Sample(1,VWC_94,FP2)
Sample(1,VWC_95,FP2)
Sample(1,VWC_96,FP2)
Sample(1,VWC_97,FP2)
Sample(1,VWC_98,FP2)
Sample(1,VWC_99,FP2)
Sample(1,VWC_100,FP2)

Sample(1,VWC_101,FP2)
Sample(1,VWC_102,FP2)
Sample(1,VWC_103,FP2)
Sample(1,VWC_104,FP2)
Sample(1,VWC_105,FP2)
Sample(1,VWC_106,FP2)
Sample(1,VWC_107,FP2)
Sample(1,VWC_108,FP2)
Sample(1,VWC_109,FP2)
Sample(1,VWC_110,FP2)
Sample(1,VWC_111,FP2)
Sample(1,VWC_112,FP2)
Sample(1,VWC_113,FP2)
Sample(1,VWC_114,FP2)
Sample(1,VWC_115,FP2)
Sample(1,VWC_116,FP2)
Sample(1,VWC_117,FP2)
Sample(1,VWC_118,FP2)
Sample(1,VWC_119,FP2)
Sample(1,VWC_120,FP2)
Sample(1,VWC_121,FP2)
Sample(1,VWC_122,FP2)
Sample(1,VWC_123,FP2)
Sample(1,VWC_124,FP2)
Sample(1,VWC_125,FP2)
Sample(1,VWC_126,FP2)
Sample(1,VWC_127,FP2)
Sample(1,VWC_128,FP2)
Sample(1,VWC_129,FP2)
Sample(1,VWC_130,FP2)
Sample(1,VWC_131,FP2)
Sample(1,VWC_132,FP2)
Sample(1,VWC_133,FP2)
Sample(1,VWC_134,FP2)
Sample(1,VWC_135,FP2)
Sample(1,VWC_136,FP2)
Sample(1,VWC_137,FP2)
Sample(1,VWC_138,FP2)
Sample(1,VWC_139,FP2)
Sample(1,VWC_140,FP2)
Sample(1,VWC_141,FP2)
Sample(1,VWC_142,FP2)
Sample(1,VWC_143,FP2)
Sample(1,VWC_144,FP2)
Sample(1, VW_1, FP2)
Sample(1,VW_2,FP2)

Sample(1,VW_3,FP2)
Sample(1,VW_4,FP2)
Sample(1,VW_5,FP2)
Sample(1,VW_6,FP2)
Sample(1,VW_7,FP2)
Sample(1,VW_8,FP2)
Sample(1,VW_9,FP2)
Sample(1,VW_10,FP2)
Sample(1,VW_11,FP2)
Sample(1,VW_12,FP2)
Sample(1,VW_13,FP2)
Sample(1,VW_14,FP2)
Sample(1,VW_15,FP2)
Sample(1,VW_16,FP2)
Sample(1,VW_17,FP2)
Sample(1,VW_18,FP2)
Sample(1,VW_19,FP2)
Sample(1,VW_20,FP2)
Sample(1,VW_21,FP2)
Sample(1,VW_22,FP2)
Sample(1,VW_23,FP2)
Sample(1,VW_24,FP2)
Sample(1,VW_25,FP2)
Sample(1,VW_26,FP2)
Sample(1,VW_27,FP2)
Sample(1,VW_28,FP2)
Sample(1,VW_29,FP2)
Sample(1,VW_30,FP2)
Sample(1,VW_31,FP2)
Sample(1,VW_32,FP2)
Sample(1,VW_33,FP2)
Sample(1,VW_34,FP2)
Sample(1,VW_35,FP2)
Sample(1,VW_36,FP2)
Sample(1,VW_37,FP2)
Sample(1,VW_38,FP2)
Sample(1,VW_39,FP2)
Sample(1,VW_40,FP2)
Sample(1,VW_41,FP2)
Sample(1,VW_42,FP2)
Sample(1,VW_43,FP2)
Sample(1,VW_44,FP2)
Sample(1,VW_45,FP2)
Sample(1,VW_46,FP2)
Sample(1,VW_47,FP2)
Sample(1,VW_48,FP2)

Sample(1,VW_49,FP2)
Sample(1,VW_50,FP2)
Sample(1,VW_51,FP2)
Sample(1,VW_52,FP2)
Sample(1,VW_53,FP2)
Sample(1,VW_54,FP2)
Sample(1,VW_55,FP2)
Sample(1,VW_56,FP2)
Sample(1,VW_57,FP2)
Sample(1,VW_58,FP2)
Sample(1,VW_59,FP2)
Sample(1,VW_60,FP2)
Sample(1,VW_61,FP2)
Sample(1,VW_62,FP2)
Sample(1,VW_63,FP2)
Sample(1,VW_64,FP2)
Sample(1,VW_65,FP2)
Sample(1,VW_66,FP2)
Sample(1,VW_67,FP2)
Sample(1,VW_68,FP2)
Sample(1,VW_69,FP2)
Sample(1,VW_70,FP2)
Sample(1,VW_71,FP2)
Sample(1,VW_72,FP2)
Sample(1,VW_73,FP2)
Sample(1,VW_74,FP2)
Sample(1,VW_75,FP2)
Sample(1,VW_76,FP2)
Sample(1,VW_77,FP2)
Sample(1,VW_78,FP2)
Sample(1,VW_79,FP2)
Sample(1,VW_80,FP2)
Sample(1,VW_81,FP2)
Sample(1,VW_82,FP2)
Sample(1,VW_83,FP2)
Sample(1,VW_84,FP2)
Sample(1,VW_85,FP2)
Sample(1,VW_86,FP2)
Sample(1,VW_87,FP2)
Sample(1,VW_88,FP2)
Sample(1,VW_89,FP2)
Sample(1,VW_90,FP2)
Sample(1,VW_91,FP2)
Sample(1,VW_92,FP2)
Sample(1,VW_93,FP2)
Sample(1,VW_94,FP2)

Sample(1,VW_95,FP2)
Sample(1,VW_96,FP2)
Sample(1,VW_97,FP2)
Sample(1,VW_98,FP2)
Sample(1,VW_99,FP2)
Sample(1,VW_100,FP2)
Sample(1,VW_101,FP2)
Sample(1,VW_102,FP2)
Sample(1,VW_103,FP2)
Sample(1,VW_104,FP2)
Sample(1,VW_105,FP2)
Sample(1,VW_106,FP2)
Sample(1,VW_107,FP2)
Sample(1,VW_108,FP2)
Sample(1,VW_109,FP2)
Sample(1,VW_110,FP2)
Sample(1,VW_111,FP2)
Sample(1,VW_112,FP2)
Sample(1,VW_113,FP2)
Sample(1,VW_114,FP2)
Sample(1,VW_115,FP2)
Sample(1,VW_116,FP2)
Sample(1,VW_117,FP2)
Sample(1,VW_118,FP2)
Sample(1,VW_119,FP2)
Sample(1,VW_120,FP2)
Sample(1,VW_121,FP2)
Sample(1,VW_122,FP2)
Sample(1,VW_123,FP2)
Sample(1,VW_124,FP2)
Sample(1,VW_125,FP2)
Sample(1,VW_126,FP2)
Sample(1,VW_127,FP2)
Sample(1,VW_128,FP2)
Sample(1,VW_129,FP2)
Sample(1,VW_130,FP2)
Sample(1,VW_131,FP2)
Sample(1,VW_132,FP2)
Sample(1,VW_133,FP2)
Sample(1,VW_134,FP2)
Sample(1,VW_135,FP2)
Sample(1,VW_136,FP2)
Sample(1,VW_137,FP2)
Sample(1,VW_138,FP2)
Sample(1,VW_139,FP2)
Sample(1,VW_140,FP2)

```
Sample(1,VW_141,FP2)
Sample(1,VW_142,FP2)
Sample(1,VW_143,FP2)
Sample(1,VW_144,FP2)
EndTable
DataTable(MPS,True,-1)
  DataInterval (0, 180, min, 10)
    Sample(1,M1_kPa,FP2)
  Sample(1,M1_DegC,FP2)
```

'by including kPa and DegC in the variable names the data will not have to be parsed into values and units later

```
Sample(1,M2_kPa,FP2)
Sample(1,M2_DegC,FP2)
Sample(1,M3_kPa,FP2)
Sample(1,M3_DegC,FP2)
Sample(1,M4_kPa,FP2)
Sample(1,M4_DegC,FP2)
Sample(1,M5_kPa,FP2)
Sample(1,M5_DegC,FP2)
Sample(1,M6_kPa,FP2)
Sample(1,M6_DegC,FP2)
Sample(1,M7_kPa,FP2)
Sample(1,M7_DegC,FP2)
Sample(1,M8_kPa,FP2)
Sample(1,M8_DegC,FP2)
Sample(1,M9_kPa,FP2)
Sample(1,M9_DegC,FP2)
Sample(1,M10_kPa,FP2)
Sample(1,M10_DegC,FP2)
Sample(1,M11_kPa,FP2)
Sample(1,M11_DegC,FP2)
Sample(1,M12_kPa,FP2)
Sample(1,M12_DegC,FP2)
Sample(1,M13_kPa,FP2)
Sample(1,M13_DegC,FP2)
Sample(1,M14_kPa,FP2)
Sample(1,M14_DegC,FP2)
Sample(1,M15_kPa,FP2)
Sample(1,M15_DegC,FP2)
Sample(1,M16_kPa,FP2)
Sample(1,M16_DegC,FP2)
Sample(1,M17_kPa,FP2)
Sample(1,M17_DegC,FP2)
Sample(1,M18_kPa,FP2)
Sample(1,M18_DegC,FP2)
Sample(1,M19_kPa,FP2)
```

Sample(1,M19_DegC,FP2)
Sample(1,M20_kPa,FP2)
Sample(1,M20_DegC,FP2)
Sample(1,M21_kPa,FP2)
Sample(1,M21_DegC,FP2)
Sample(1,M22_kPa,FP2)
Sample(1,M22_DegC,FP2)
Sample(1,M23_kPa,FP2)
Sample(1,M23_DegC,FP2)
Sample(1,M24_kPa,FP2)
Sample(1,M24_DegC,FP2)
Sample(1,M25_kPa,FP2)
Sample(1,M25_DegC,FP2)
Sample(1,M26_kPa,FP2)
Sample(1,M26_DegC,FP2)
Sample(1,M27_kPa,FP2)
Sample(1,M27_DegC,FP2)
Sample(1,M28_kPa,FP2)
Sample(1,M28_DegC,FP2)
Sample(1,M29_kPa,FP2)
Sample(1,M29_DegC,FP2)
Sample(1,M30_kPa,FP2)
Sample(1,M30_DegC,FP2)
Sample(1,M31_kPa,FP2)
Sample(1,M31_DegC,FP2)
Sample(1,M32_kPa,FP2)
Sample(1,M32_DegC,FP2)
Sample(1,M33_kPa,FP2)
Sample(1,M33_DegC,FP2)
Sample(1,M34_kPa,FP2)
Sample(1,M34_DegC,FP2)
Sample(1,M35_kPa,FP2)
Sample(1,M35_DegC,FP2)
Sample(1,M36_kPa,FP2)
Sample(1,M36_DegC,FP2)
Sample(1,M37_kPa,FP2)
Sample(1,M37_DegC,FP2)
Sample(1,M38_kPa,FP2)
Sample(1,M38_DegC,FP2)
Sample(1,M39_kPa,FP2)
Sample(1,M39_DegC,FP2)
Sample(1,M40_kPa,FP2)
Sample(1,M40_DegC,FP2)
Sample(1,M41_kPa,FP2)
Sample(1,M41_DegC,FP2)
Sample(1,M42_kPa,FP2)

```

Sample(1,M42_DegC,FP2)
Sample(1,M43_kPa,FP2)
Sample(1,M43_DegC,FP2)
Sample(1,M44_kPa,FP2)
Sample(1,M44_DegC,FP2)
Sample(1,M45_kPa,FP2)
Sample(1,M45_DegC,FP2)
Sample(1,M46_kPa,FP2)
Sample(1,M46_DegC,FP2)
Sample(1,M47_kPa,FP2)
Sample(1,M47_DegC,FP2)
Sample(1,M48_kPa,FP2)
Sample(1,M48_DegC,FP2)
Sample(1,M49_kPa,FP2)
Sample(1,M49_DegC,FP2)
Sample(1,M50_kPa,FP2)
Sample(1,M50_DegC,FP2)
Sample(1,M51_kPa,FP2)
Sample(1,M51_DegC,FP2)
Sample(1,M52_kPa,FP2)
Sample(1,M52_DegC,FP2)
Sample(1,M53_kPa,FP2)
Sample(1,M53_DegC,FP2)
Sample(1,M54_kPa,FP2)
Sample(1,M54_DegC,FP2)
End Table
DataTable(Table2,True,-1)
    DataInterval(0,1440,Min,10)
    Minimum(1,BattV,FP2,False,False)
EndTable

BeginProg
Scan(180,min,1,0) 'First number changed to modify scan interval
    Battery(BattV)
    PanelTemp(PTemp_C,_60Hz)
'Turn AM1632B Multiplexer which is hooked into Com Port 2, Differential Channel 2 on
    PortSet(2,1)
    Delay(0,150,mSec)
    LCount145=1
    SubScan(0,uSec,16)
    PulsePort(1,10000) 'the first number is the Com Port which has the CLK wire
'Full Bridge measurements of Tensiometers connected to Differential Channel 1 and VX1
BrFull(T_1(LCount145),1,mv25,1,1,1,2500,True,True,0,_60Hz,Mult(LCount145),Offs(LCount1
45))
LCount145=LCount145+1
    NextSubScan

```


'Turn Com Port 2 off

PortSet(2,0)
Delay(0,150,mSec)
PortSet(4,1)
Delay(0,150,mSec)
LCount146=1
SubScan(0,uSec,16)
PulsePort(1,10000)

BrFull(T_2(LCount146),1,mv25,2,1,1,2500,True,True,0,_60Hz,Mult_2(LCount146),Offs_2(LCount146))

LCount146=LCount146+1
NextSubScan
PortSet(4,0)
Delay(0,150,mSec)

'Convert raw values of T(1-16) and T_2(17-32) to kPa

T_kPa_1 = (T_1(1)*79.35+56.02)/10
T_kPa_2 = (T_1(2)*79.35+56.02)/10
T_kPa_3 = (T_1(3)*79.35+56.02)/10
T_kPa_4 = (T_1(4)*79.35+56.02)/10
T_kPa_5 = (T_1(5)*79.35+56.02)/10
T_kPa_6 = (T_1(6)*79.35+56.02)/10
T_kPa_7 = (T_1(7)*79.35+56.02)/10
T_kPa_8 = (T_1(8)*79.35+56.02)/10
T_kPa_9 = (T_1(9)*79.35+56.02)/10
T_kPa_10 = (T_1(10)*79.35+56.02)/10
T_kPa_11 = (T_1(11)*79.35+56.02)/10
T_kPa_12 = (T_1(12)*79.35+56.02)/10
T_kPa_13 = (T_1(13)*79.35+56.02)/10
T_kPa_14 = (T_1(14)*79.35+56.02)/10
T_kPa_15 = (T_1(15)*79.35+56.02)/10
T_kPa_16 = (T_1(16)*79.35+56.02)/10
T_kPa_17 = (T_2(1)*79.35+56.02)/10
T_kPa_18 = (T_2(2)*79.35+56.02)/10
T_kPa_19 = (T_2(3)*79.35+56.02)/10
T_kPa_20 = (T_2(4)*79.35+56.02)/10
T_kPa_21 = (T_2(5)*79.35+56.02)/10
T_kPa_22 = (T_2(6)*79.35+56.02)/10
T_kPa_23 = (T_2(7)*79.35+56.02)/10
T_kPa_24 = (T_2(8)*79.35+56.02)/10
T_kPa_25 = (T_2(9)*79.35+56.02)/10
T_kPa_26 = (T_2(10)*79.35+56.02)/10
T_kPa_27 = (T_2(11)*79.35+56.02)/10
T_kPa_28 = (T_2(12)*79.35+56.02)/10
T_kPa_29 = (T_2(13)*79.35+56.02)/10
T_kPa_30 = (T_2(14)*79.35+56.02)/10
T_kPa_31 = (T_2(15)*79.35+56.02)/10

T_kPa_32 = (T_2(16)*79.35+56.02)/10

CallTable Tens

'EC-5 Measurements

'Turn AM1632B hooked into Com7 and Single Ended Channels 10-12 on

PortSet(7,1)
Delay(0,150,mSec)
'Pulse CLK- in Com 1
PulsePort(1,10000)

'ECHO Probe EC-5 measurement VW_49 on the AM16/32 Multiplexer

BrHalf(VW_49,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)

'specifying the multiplier of 2500 and the offset of 0 gives the raw mv output

BrHalf(VW_50,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_51,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)

'Switch to next AM16/32 Multiplexer channel

PulsePort(1,10000)
BrHalf(VW_52,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_53,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_54,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_55,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_56,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_57,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_58,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_59,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_60,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_61,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_62,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_63,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_64,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_65,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_66,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_67,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_68,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_69,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_70,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_71,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_72,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_73,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_74,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)

```

BrHalf(VW_75,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_76,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_77,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_78,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_79,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_80,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_81,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_82,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_83,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_84,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_85,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_86,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_87,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_88,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_89,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_90,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_91,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_92,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_93,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_94,1,mV2500,10,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_95,1,mV2500,11,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_96,1,mV2500,12,1,1,2500,False,10000,_60Hz,2500,0)
'Turn off Com 7
PortSet(7,0)
Delay(0,150,mSec)

```

'Turn AM16/32 Multiplexer On in Com 8 and single ended channels 13-15

```

PortSet(8,1)
Delay(0,150,mSec)
PulsePort(1,10000)
BrHalf(VW_97,1,mV2500,13,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_98,1,mV2500,14,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_99,1,mV2500,15,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_100,1,mV2500,13,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_101,1,mV2500,14,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_102,1,mV2500,15,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_103,1,mV2500,13,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_104,1,mV2500,14,1,1,2500,False,10000,_60Hz,2500,0)

```


BrHalf(VW_139,1,mV2500,13,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_140,1,mV2500,14,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_141,1,mV2500,15,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_142,1,mV2500,13,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_143,1,mV2500,14,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_144,1,mV2500,15,1,1,2500,False,10000,_60Hz,2500,0)

'Turn Com 8 off

PortSet(8,0)
Delay(0,150,mSec)

'Turn AM1632B wired into Com Port 6 and Single Ended Channels 7-9

PortSet(6,1)
Delay(0,150,mSec)
PulsePort(1,10000)
BrHalf(VW_1,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_2,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_3,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_4,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_5,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_6,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_7,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_8,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_9,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_10,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_11,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_12,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_13,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_14,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_15,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_16,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_17,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_18,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_19,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_20,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_21,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_22,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_23,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_24,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)

```

BrHalf(VW_25,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_26,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_27,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_28,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_29,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_30,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_31,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_32,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_33,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_34,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_35,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_36,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_37,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_38,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_39,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_40,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_41,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_42,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_43,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_44,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_45,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)
PulsePort(1,10000)
BrHalf(VW_46,1,mV2500,7,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_47,1,mV2500,8,1,1,2500,False,10000,_60Hz,2500,0)
BrHalf(VW_48,1,mV2500,9,1,1,2500,False,10000,_60Hz,2500,0)

```

'Turn AM16/32 Multiplexer Off

```

PortSet(6,0)
Delay(0,150,mSec)

```

'convert raw millivolt VW output into VWC with the calibration equation developed

```

VWC_1=VW_1*.0016-.473
VWC_2=VW_2*.0016-.473
VWC_3=VW_3*.0016-.473
VWC_4=VW_4*.0016-.473
VWC_5=VW_5*.0016-.473
VWC_6=VW_6*.0016-.473
VWC_7=VW_7*.0016-.473
VWC_8=VW_8*.0016-.473
VWC_9=VW_9*.0016-.473
VWC_10=VW_10*.0016-.473

```

VWC_11=VW_11*.0016-.473
VWC_12=VW_12*.0016-.473
VWC_13=VW_13*.0016-.473
VWC_14=VW_14*.0016-.473
VWC_15=VW_15*.0016-.473
VWC_16=VW_16*.0016-.473
VWC_17=VW_17*.0016-.473
VWC_18=VW_18*.0016-.473
VWC_19=VW_19*.0016-.473
VWC_20=VW_20*.0016-.473
VWC_21=VW_21*.0016-.473
VWC_22=VW_22*.0016-.473
VWC_23=VW_23*.0016-.473
VWC_24=VW_24*.0016-.473
VWC_25=VW_25*.0016-.473
VWC_26=VW_26*.0016-.473
VWC_27=VW_27*.0016-.473
VWC_28=VW_28*.0016-.473
VWC_29=VW_29*.0016-.473
VWC_30=VW_30*.0016-.473
VWC_31=VW_31*.0016-.473
VWC_32=VW_32*.0016-.473
VWC_33=VW_33*.0016-.473
VWC_34=VW_34*.0016-.473
VWC_35=VW_35*.0016-.473
VWC_36=VW_36*.0016-.473
VWC_37=VW_37*.0016-.473
VWC_38=VW_38*.0016-.473
VWC_39=VW_39*.0016-.473
VWC_40=VW_40*.0016-.473
VWC_41=VW_41*.0016-.473
VWC_42=VW_42*.0016-.473
VWC_43=VW_43*.0016-.473
VWC_44=VW_44*.0016-.473
VWC_45=VW_45*.0016-.473
VWC_46=VW_46*.0016-.473
VWC_47=VW_47*.0016-.473
VWC_48=VW_48*.0016-.473
VWC_49=VW_49*.0016-.473
VWC_50=VW_50*.0016-.473
VWC_51=VW_51*.0016-.473
VWC_52=VW_52*.0016-.473
VWC_53=VW_53*.0016-.473
VWC_54=VW_54*.0016-.473
VWC_55=VW_55*.0016-.473
VWC_56=VW_56*.0016-.473

VWC_57=VW_57*.0016-.473
VWC_58=VW_58*.0016-.473
VWC_59=VW_59*.0016-.473
VWC_60=VW_60*.0016-.473
VWC_61=VW_61*.0016-.473
VWC_62=VW_62*.0016-.473
VWC_63=VW_63*.0016-.473
VWC_64=VW_64*.0016-.473
VWC_65=VW_65*.0016-.473
VWC_66=VW_66*.0016-.473
VWC_67=VW_67*.0016-.473
VWC_68=VW_68*.0016-.473
VWC_69=VW_69*.0016-.473
VWC_70=VW_70*.0016-.473
VWC_71=VW_71*.0016-.473
VWC_72=VW_72*.0016-.473
VWC_73=VW_73*.0016-.473
VWC_74=VW_74*.0016-.473
VWC_75=VW_75*.0016-.473
VWC_76=VW_76*.0016-.473
VWC_77=VW_77*.0016-.473
VWC_78=VW_78*.0016-.473
VWC_79=VW_79*.0016-.473
VWC_80=VW_80*.0016-.473
VWC_81=VW_81*.0016-.473
VWC_82=VW_82*.0016-.473
VWC_83=VW_83*.0016-.473
VWC_84=VW_84*.0016-.473
VWC_85=VW_85*.0016-.473
VWC_86=VW_86*.0016-.473
VWC_87=VW_87*.0016-.473
VWC_88=VW_88*.0016-.473
VWC_89=VW_89*.0016-.473
VWC_90=VW_90*.0016-.473
VWC_91=VW_91*.0016-.473
VWC_92=VW_92*.0016-.473
VWC_93=VW_93*.0016-.473
VWC_94=VW_94*.0016-.473
VWC_95=VW_95*.0016-.473
VWC_96=VW_96*.0016-.473
VWC_97=VW_97*.0016-.473
VWC_98=VW_98*.0016-.473
VWC_99=VW_99*.0016-.473
VWC_100=VW_100*.0016-.473
VWC_101=VW_101*.0016-.473
VWC_102=VW_102*.0016-.473

VWC_103=VW_103*.0016-.473
VWC_104=VW_104*.0016-.473
VWC_105=VW_105*.0016-.473
VWC_106=VW_106*.0016-.473
VWC_107=VW_107*.0016-.473
VWC_108=VW_108*.0016-.473
VWC_109=VW_109*.0016-.473
VWC_110=VW_110*.0016-.473
VWC_111=VW_111*.0016-.473
VWC_112=VW_112*.0016-.473
VWC_113=VW_113*.0016-.473
VWC_114=VW_114*.0016-.473
VWC_115=VW_115*.0016-.473
VWC_116=VW_116*.0016-.473
VWC_117=VW_117*.0016-.473
VWC_118=VW_118*.0016-.473
VWC_119=VW_119*.0016-.473
VWC_120=VW_120*.0016-.473
VWC_121=VW_121*.0016-.473
VWC_122=VW_122*.0016-.473
VWC_123=VW_123*.0016-.473
VWC_124=VW_124*.0016-.473
VWC_125=VW_125*.0016-.473
VWC_126=VW_126*.0016-.473
VWC_127=VW_127*.0016-.473
VWC_128=VW_128*.0016-.473
VWC_129=VW_129*.0016-.473
VWC_130=VW_130*.0016-.473
VWC_131=VW_131*.0016-.473
VWC_132=VW_132*.0016-.473
VWC_133=VW_133*.0016-.473
VWC_134=VW_134*.0016-.473
VWC_135=VW_135*.0016-.473
VWC_136=VW_136*.0016-.473
VWC_137=VW_137*.0016-.473
VWC_138=VW_138*.0016-.473
VWC_139=VW_139*.0016-.473
VWC_140=VW_140*.0016-.473
VWC_141=VW_141*.0016-.473
VWC_142=VW_142*.0016-.473
VWC_143=VW_143*.0016-.473
VWC_144=VW_144*.0016-.473

CallTable VWC

'SDI12 measurements of MPS-6 sensors in Com Port 3

Move(SDI12_1(),2,NaN,1)

SDI12Recorder(SDI12_1(),3,"a","M!",1,0)
Move(SDI12_2(),2,NaN,1)
SDI12Recorder(SDI12_2(),3,"b","M!",1,0)
Move(SDI12_3(),2,NaN,1)
SDI12Recorder(SDI12_3(),3,"c","M!",1,0)
Move(SDI12_4(),2,NaN,1)
SDI12Recorder(SDI12_4(),3,"d","M!",1,0)
Move(SDI12_5(),2,NaN,1)
SDI12Recorder(SDI12_5(),3,"e","M!",1,0)
Move(SDI12_6(),2,NaN,1)
SDI12Recorder(SDI12_6(),3,"f","M!",1,0)
Move(SDI12_7(),2,NaN,1)
SDI12Recorder(SDI12_7(),3,"g","M!",1,0)
Move(SDI12_8(),2,NaN,1)
SDI12Recorder(SDI12_8(),3,"h","M!",1,0)
Move(SDI12_9(),2,NaN,1)
SDI12Recorder(SDI12_9(),3,"i","M!",1,0)
Move(SDI12_10(),2,NaN,1)
SDI12Recorder(SDI12_10(),3,"j","M!",1,0)
Move(SDI12_11(),2,NaN,1)
SDI12Recorder(SDI12_11(),3,"k","M!",1,0)
Move(SDI12_12(),2,NaN,1)
SDI12Recorder(SDI12_12(),3,"l","M!",1,0)
Move(SDI12_13(),2,NaN,1)
SDI12Recorder(SDI12_13(),3,"m","M!",1,0)
Move(SDI12_14(),2,NaN,1)
SDI12Recorder(SDI12_14(),3,"n","M!",1,0)
Move(SDI12_15(),2,NaN,1)
SDI12Recorder(SDI12_15(),3,"o","M!",1,0)
Move(SDI12_16(),2,NaN,1)
SDI12Recorder(SDI12_16(),3,"p","M!",1,0)
Move(SDI12_17(),2,NaN,1)
SDI12Recorder(SDI12_17(),3,"q","M!",1,0)
Move(SDI12_18(),2,NaN,1)
SDI12Recorder(SDI12_18(),3,"r","M!",1,0)
Move(SDI12_19(),2,NaN,1)
SDI12Recorder(SDI12_19(),3,"s","M!",1,0)
Move(SDI12_20(),2,NaN,1)
SDI12Recorder(SDI12_20(),3,"t","M!",1,0)
Move(SDI12_21(),2,NaN,1)
SDI12Recorder(SDI12_21(),3,"u","M!",1,0)
Move(SDI12_22(),2,NaN,1)
SDI12Recorder(SDI12_22(),3,"v","M!",1,0)
Move(SDI12_23(),2,NaN,1)
SDI12Recorder(SDI12_23(),3,"w","M!",1,0)
Move(SDI12_24(),2,NaN,1)

```

SDI12Recorder(SDI12_24(),3,"x","M!",1,0)
Move(SDI12_25(),2,NaN,1)
SDI12Recorder(SDI12_25(),3,"y","M!",1,0)
Move(SDI12_26(),2,NaN,1)
SDI12Recorder(SDI12_26(),3,"z","M!",1,0)
Move(SDI12_27(),2,NaN,1)
SDI12Recorder(SDI12_27(),3,"A","M!",1,0)
'continue measuring MPS-6 sensors on Com Port 5
Move(SDI12_28(),2,NaN,1)
SDI12Recorder(SDI12_28(),5,"B","M!",1,0)
Move(SDI12_29(),2,NaN,1)
SDI12Recorder(SDI12_29(),5,"C","M!",1,0)
Move(SDI12_30(),2,NaN,1)
SDI12Recorder(SDI12_30(),5,"D","M!",1,0)
Move(SDI12_31(),2,NaN,1)
SDI12Recorder(SDI12_31(),5,"E","M!",1,0)
Move(SDI12_32(),2,NaN,1)
SDI12Recorder(SDI12_32(),5,"F","M!",1,0)
Move(SDI12_33(),2,NaN,1)
SDI12Recorder(SDI12_33(),5,"G","M!",1,0)
Move(SDI12_34(),2,NaN,1)
SDI12Recorder(SDI12_34(),5,"H","M!",1,0)
Move(SDI12_35(),2,NaN,1)
SDI12Recorder(SDI12_35(),5,"I","M!",1,0)
Move(SDI12_36(),2,NaN,1)
SDI12Recorder(SDI12_36(),5,"J","M!",1,0)
Move(SDI12_37(),2,NaN,1)
SDI12Recorder(SDI12_37(),5,"K","M!",1,0)
Move(SDI12_38(),2,NaN,1)
SDI12Recorder(SDI12_38(),5,"L","M!",1,0)
Move(SDI12_39(),2,NaN,1)
SDI12Recorder(SDI12_39(),5,"M","M!",1,0)
Move(SDI12_40(),2,NaN,1)
SDI12Recorder(SDI12_40(),5,"N","M!",1,0)
Move(SDI12_41(),2,NaN,1)
SDI12Recorder(SDI12_41(),5,"O","M!",1,0)
Move(SDI12_42(),2,NaN,1)
SDI12Recorder(SDI12_42(),5,"P","M!",1,0)
Move(SDI12_43(),2,NaN,1)
SDI12Recorder(SDI12_43(),5,"Q","M!",1,0)
Move(SDI12_44(),2,NaN,1)
SDI12Recorder(SDI12_44(),5,"R","M!",1,0)
Move(SDI12_45(),2,NaN,1)
SDI12Recorder(SDI12_45(),5,"S","M!",1,0)
Move(SDI12_46(),2,NaN,1)
SDI12Recorder(SDI12_46(),5,"T","M!",1,0)

```

```
Move(SDI12_47(),2,NaN,1)
SDI12Recorder(SDI12_47(),5,"U","M!",1,0)
Move(SDI12_48(),2,NaN,1)
SDI12Recorder(SDI12_48(),5,"V","M!",1,0)
Move(SDI12_49(),2,NaN,1)
SDI12Recorder(SDI12_49(),5,"W","M!",1,0)
Move(SDI12_50(),2,NaN,1)
SDI12Recorder(SDI12_50(),5,"X","M!",1,0)
Move(SDI12_51(),2,NaN,1)
SDI12Recorder(SDI12_51(),5,"Y","M!",1,0)
Move(SDI12_52(),2,NaN,1)
SDI12Recorder(SDI12_52(),5,"Z","M!",1,0)
Move(SDI12_53(),2,NaN,1)
SDI12Recorder(SDI12_53(),5,"1","M!",1,0)
Move(SDI12_54(),2,NaN,1)
SDI12Recorder(SDI12_54(),5,"2","M!",1,0)
```

```
CallTable MPS
CallTable Table2
NextScan
EndProg
```

Appendix C - CR1000 (2) Programming (Tensiometers 33-54 and Automatic Irrigation System)

'Declare Variables and Units

Public VWC_1
Public VWC_5
Public VWC_9
Public VWC_13
Public VWC_17
Public VWC_21
Public VWC_25
Public VWC_29
Public VWC_33
Public VWC_37
Public VWC_41
Public VWC_45
Public VWC_49
Public VWC_53
Public VWC_57
Public VWC_61
Public VWC_65
Public VWC_69
Public VWC_73
Public VWC_77
Public VWC_81
Public VWC_85
Public VWC_89
Public VWC_93
Public VWC_97
Public VWC_101
Public VWC_105
Public VWC_109
Public VWC_113
Public VWC_117
Public VWC_121
Public VWC_125
Public VWC_129
Public VWC_133
Public VWC_137
Public VWC_141
Public MPS1

Public MPS3
Public MPS5
Public MPS7
Public MPS9
Public MPS11
Public MPS13
Public MPS15
Public MPS17
Public MPS19
Public MPS21
Public MPS23
Public MPS25
Public MPS27
Public MPS29
Public MPS31
Public MPS33
Public MPS35
Public BattV
Public PTemp_C
Public LCount
Public LCount2
Public FullBR_3(16
Public FullBR(6)
Public Mult(6)={1,1,1,1,1,1}
Public Offs(6)={0,0,0,0,0,0}
Public Mult_3(16)={1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1}
Public Offs_3(16)={0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0}
Public ResultCode
Public ValveCtrl(48)
Units BattV=Volts
Units PTemp_C=Deg C
Units FullBR=mV/V
Units FullBR_3=mV/V
Public T_kPa_33
Public T_kPa_34
Public T_kPa_35
Public T_kPa_36
Public T_kPa_37
Public T_kPa_38
Public T_kPa_39
Public T_kPa_40
Public T_kPa_41
Public T_kPa_42
Public T_kPa_43
Public T_kPa_44
Public T_kPa_45

Public T_kPa_46
Public T_kPa_47
Public T_kPa_48
Public T_kPa_49
Public T_kPa_50
Public T_kPa_51
Public T_kPa_52
Public T_kPa_53
Public T_kPa_54

'Define Data Tables

DataTable(Tens2,True,-1)
 DataInterval(0,360,min, 10)

Sample(1,T_kPa_33,FP2)
Sample(1,T_kPa_34,FP2)
Sample(1,T_kPa_35,FP2)
Sample(1,T_kPa_36,FP2)
Sample(1,T_kPa_37,FP2)
Sample(1,T_kPa_38,FP2)
Sample(1,T_kPa_39,FP2)
Sample(1,T_kPa_40,FP2)
Sample(1,T_kPa_41,FP2)
Sample(1,T_kPa_42,FP2)
Sample(1,T_kPa_43,FP2)
Sample(1,T_kPa_44,FP2)
Sample(1,T_kPa_45,FP2)
Sample(1,T_kPa_46,FP2)
Sample(1,T_kPa_47,FP2)
Sample(1,T_kPa_48,FP2)
Sample(1,T_kPa_49,FP2)
Sample(1,T_kPa_50,FP2)
Sample(1,T_kPa_51,FP2)
Sample(1,T_kPa_52,FP2)
Sample(1,T_kPa_53,FP2)
Sample(1,T_kPa_54,FP2)

Sample(1,FullBR_3(1),FP2)
 Sample(1,FullBR_3(2),FP2)
 Sample(1,FullBR_3(3),FP2)
 Sample(1,FullBR_3(4),FP2)
 Sample(1,FullBR_3(5),FP2)
 Sample(1,FullBR_3(6),FP2)
 Sample(1,FullBR_3(7),FP2)
 Sample(1,FullBR_3(8),FP2)
 Sample(1,FullBR_3(9),FP2)

```

Sample(1,FullBR_3(10),FP2)
Sample(1,FullBR_3(11),FP2)
Sample(1,FullBR_3(12),FP2)
Sample(1,FullBR_3(13),FP2)
Sample(1,FullBR_3(14),FP2)
Sample(1,FullBR_3(15),FP2)
Sample(1,FullBR_3(16),FP2)
Sample(1,FullBR(1),FP2)
Sample(1,FullBR(2),FP2)
Sample(1,FullBR(3),FP2)
Sample(1,FullBR(4),FP2)
Sample(1,FullBR(5),FP2)
Sample(1,FullBR(6),FP2)

```

EndTable

```

DataTable(Table2,True,-1)
  DataInterval(0,1440,Min,10)
  Minimum(1,BattV,FP2,False,False)
EndTable

```

```

DataTable (WateringRecord, True, -1)
  DataInterval (0,60, Min, 10) 'change back to 480 for an 8 hour scan interval which will
record the watering status at 8am
  Sample (48, ValveCtrl(), FP2) 'change first number for number of repetitions
EndTable

```

```

'Main Program
BeginProg
  'Main Scan
  Scan(1,min,1,0)'change scan to five minutes for a program that waters for five
minutes to ensure proper start and stop
  'Default Datalogger Battery Voltage measurement 'BattV'
  Battery(BattV)
  'Default Wiring Panel Temperature measurement 'PTemp_C'
  PanelTemp(PTemp_C,_60Hz)
  'Turn AM16/32 Multiplexer On
  PortSet(8,1)
  Delay(0,150,mSec)
  LCount=1
  SubScan(0,uSec,6)
  'Switch to next AM16/32 Multiplexer channel
  PulsePort(4,10000)
  'Generic Full Bridge measurements 'FullBR()' on the AM16/32

```

Multiplexer

BrFull(FullBR(LCount),1,mv25,2,1,1,2500,True,True,0,_60Hz,Mult(LCount),Offs(LCount))

 LCount=LCount+1
 NextSubScan
 'Turn AM16/32 Multiplexer Off
 PortSet(8,0)
 Delay(0,150,mSec)

PortSet(7,1)

 Delay(0,150,mSec)
 LCount2=1
 SubScan(0,uSec,16)
 'Switch to next AM16/32 Multiplexer channel
 PulsePort(4,10000)
 'Generic Full Bridge measurements 'FullBR_3()' on the AM16/32

Multiplexer

BrFull(FullBR_3(LCount2),1,mv25,1,1,1,2500,True,True,0,_60Hz,Mult_3(LCount2),Offs_3(LCount2))

 LCount2=LCount2+1
 NextSubScan
 'Turn AM16/32 Multiplexer Off
 PortSet(7,0)
 Delay(0,150,mSec)

The GetVariables commands will collect EC-5 and MPS data from the specified sensors, to be used in making the automatic irrigation decisions

GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_1",VWC_1,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_5",VWC_5,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_9",VWC_9,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_13",VWC_13,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_17",VWC_17,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_21",VWC_21,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_25",VWC_25,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_29",VWC_29,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_33",VWC_33,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_37",VWC_37,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_41",VWC_41,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_45",VWC_45,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_49",VWC_49,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_53",VWC_53,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_57",VWC_57,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_61",VWC_61,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_65",VWC_65,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_69",VWC_69,1)

GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_73",VWC_73,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_77",VWC_77,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_81",VWC_81,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_85",VWC_85,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_89",VWC_89,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_93",VWC_93,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_97",VWC_97,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_101",VWC_101,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_105",VWC_105,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_109",VWC_109,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_113",VWC_113,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_117",VWC_117,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_121",VWC_121,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_125",VWC_125,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_129",VWC_129,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_133",VWC_133,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_137",VWC_137,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_141",VWC_141,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M1_kPa",MPS1,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M4_kPa",MPS3,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M7_kPa",MPS5,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M10_kPa",MPS7,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M13_kPa",MPS9,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M16_kPa",MPS11,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M19_kPa",MPS13,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M22_kPa",MPS15,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M25_kPa",MPS17,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M28_kPa",MPS19,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M31_kPa",MPS21,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M34_kPa",MPS23,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M37_kPa",MPS25,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M40_kPa",MPS27,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M43_kPa",MPS29,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M46_kPa",MPS31,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M49_kPa",MPS33,1)
 GetVariables (ResultCode,Com3,0,1,0000,5,"MPS","M52_kPa",MPS35,1)

If IfTime (479, 1440, Min) Then ValveCtrl(48)=1 'turn on fertilizer tank pump 1 minutes before watering scheduled

If IfTime(480, 1440,Min)AND VWC_1<0.38 AND VWC_1>0.1 Then ValveCtrl(1)=1

If IfTime(480,1440,Min) AND VWC_9<0.38 AND VWC_9>0.1 Then ValveCtrl(2)=1

If IfTime(480,1440,Min) AND VWC_17<0.38 AND VWC_17>0.1 Then ValveCtrl(3)=1

If IfTime(480,1440,Min) AND VWC_25<0.38 AND VWC_25>0.1 Then ValveCtrl(4)=1

If IfTime(480,1440,Min) AND VWC_33<0.38 AND VWC_33>0.1 Then ValveCtrl(5)=1

If IfTime(480,1440,Min) AND VWC_41<0.38 AND VWC_41>0.1 Then ValveCtrl(6)=1

If IfTime(480,1440,Min) AND VWC_49<0.38 AND VWC_49>0.1 Then ValveCtrl(7)=1
If IfTime(480,1440,Min) AND VWC_57<0.38 AND VWC_57>0.1 Then ValveCtrl(8)=1
If IfTime(480,1440,Min) AND VWC_65<0.38 AND VWC_65>0.1 Then ValveCtrl(9)=1
If IfTime(480,1440,Min) AND VWC_73<0.38 AND VWC_73>0.1 Then ValveCtrl(10)=1
If IfTime(480,1440,Min) AND VWC_81<0.38 AND VWC_81>0.1 Then ValveCtrl(11)=1
If IfTime(480,1440,Min) AND VWC_89<0.38 AND VWC_89>0.1 Then ValveCtrl(12)=1
If IfTime(480,1440,Min) AND VWC_97<0.38 AND VWC_97>0.1 Then ValveCtrl(13)=1
If IfTime(480,1440,Min) AND VWC_105<0.38 AND VWC_105>0.1 Then ValveCtrl(14)=1
If IfTime(480,1440,Min) AND VWC_113<0.38 AND VWC_113>0.1 Then ValveCtrl(15)=1
If IfTime(480,1440,Min) AND VWC_121<0.38 AND VWC_121>0.1 Then ValveCtrl(16)=1
If IfTime(480,1440,Min) AND VWC_129<0.38 AND VWC_129>0.1 Then ValveCtrl(17)=1
If IfTime(480,1440,Min) AND VWC_137<0.38 AND VWC_137>0.1 Then ValveCtrl(18)=1

If IfTime (480, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1
If IfTime (480, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (480, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (480, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (480, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (480, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (480, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (480, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (480, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (480, 1440, Min) AND MPS19 <-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (480, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (480, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (480, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (480, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (480, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (480, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (480, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (480, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1

If IfTime (483, 1440, Min) AND MPS1<-750 AND MPS1<-10 Then ValveCtrl(1)=1
If IfTime (483, 1440, Min) AND MPS3<-750 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (483, 1440, Min) AND MPS5 <-750 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (483, 1440, Min) AND MPS7<-750 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (483, 1440, Min) AND MPS9<-750 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (483, 1440, Min) AND MPS11<-750 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (483, 1440, Min) AND MPS13<-750 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (483, 1440, Min) AND MPS15 <-750 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (483, 1440, Min) AND MPS17 <-750 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (483, 1440, Min) AND MPS19 <-750 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (483, 1440, Min) AND MPS21 <-750 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (483, 1440, Min) AND MPS23 <-750 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (483, 1440, Min) AND MPS25 <-750 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (483, 1440, Min) AND MPS27 <-750 AND MPS27<-10 Then ValveCtrl(14)=1

If IfTime (483, 1440, Min) AND MPS29 <-750 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (483, 1440, Min) AND MPS31 <-750 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (483, 1440, Min) AND MPS33 <-750 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (483, 1440, Min) AND MPS35 <-750 AND MPS35<-10 Then ValveCtrl(18)=1

If IfTime (1259, 1440, Min) Then ValveCtrl(48)=1
If IfTime (1260, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1
If IfTime (1260, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (1260, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (1260, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (1260, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (1260, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (1260, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (1260, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (1260, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (1260, 1440, Min) AND MPS19<-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (1260, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (1260, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (1260, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (1260, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (1260, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (1260, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (1260, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (1260, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1

If IfTime(1260,1440,Min) AND VWC_5<0.38 AND VWC_5>0.1 Then ValveCtrl(19)=1
If IfTime(1260,1440,Min) AND VWC_13<0.38 AND VWC_13>0.1 Then ValveCtrl(20)=1
If IfTime(1260,1440,Min) AND VWC_21<0.38 AND VWC_21>0.1 Then ValveCtrl(21)=1
If IfTime(1260,1440,Min) AND VWC_29<0.38 AND VWC_29>0.1 Then ValveCtrl(22)=1
If IfTime(1260,1440,Min) AND VWC_37<0.38 AND VWC_37>0.1 Then ValveCtrl(23)=1
If IfTime(1260,1440,Min) AND VWC_45<0.38 AND VWC_45>0.1 Then ValveCtrl(24)=1
If IfTime(1260,1440,Min) AND VWC_53<0.38 AND VWC_53>0.1 Then ValveCtrl(25)=1
If IfTime(1260,1440,Min) AND VWC_61<0.38 AND VWC_61>0.1 Then ValveCtrl(26)=1
If IfTime(1260,1440,Min) AND VWC_69<0.38 AND VWC_69>0.1 Then ValveCtrl(27)=1
If IfTime(1260,1440,Min) AND VWC_77<0.38 AND VWC_77>0.1 Then ValveCtrl(28)=1
If IfTime(1260,1440,Min) AND VWC_85<0.38 AND VWC_85>0.1 Then ValveCtrl(29)=1
If IfTime(1260,1440,Min) AND VWC_93<0.38 AND VWC_93>0.1 Then ValveCtrl(30)=1
If IfTime(1260,1440,Min) AND VWC_101<0.38 AND VWC_101>0.1 Then ValveCtrl(31)=1
If IfTime(1260,1440,Min) AND VWC_109<0.38 AND VWC_109>0.1 Then ValveCtrl(32)=1
If IfTime(1260,1440,Min) AND VWC_117<0.38 AND VWC_117>0.1 Then ValveCtrl(33)=1
If IfTime(1260,1440,Min) AND VWC_125<0.38 AND VWC_125>0.1 Then ValveCtrl(34)=1
If IfTime(1260,1440,Min) AND VWC_133<0.38 AND VWC_133>0.1 Then ValveCtrl(35)=1
If IfTime(1260,1440,Min) AND VWC_141<0.38 AND VWC_141>0.1 Then ValveCtrl(36)=1

If IfTime (899, 1440, Min) Then ValveCtrl(48)=1
If IfTime (900, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1

If IfTime (900, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (900, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (900, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (900, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (900, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (900, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (900, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (900, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (900, 1440, Min) AND MPS19<-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (900, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (900, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (900, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (900, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (900, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (900, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (900, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (900, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1

If IfTime (482,1440,Min) Then ValveCtrl(1)=0
If IfTime (482,1440,Min) Then ValveCtrl(2)=0
If IfTime (482,1440,Min) Then ValveCtrl(3)=0
If IfTime (482,1440,Min) Then ValveCtrl(4)=0
If IfTime (482,1440,Min) Then ValveCtrl(5)=0
If IfTime (482,1440,Min) Then ValveCtrl(6)=0
If IfTime (482,1440,Min) Then ValveCtrl(7)=0
If IfTime (482,1440,Min) Then ValveCtrl(8)=0
If IfTime (482,1440,Min) Then ValveCtrl(9)=0
If IfTime (482,1440,Min) Then ValveCtrl(10)=0
If IfTime (482,1440,Min) Then ValveCtrl(11)=0
If IfTime (482,1440,Min) Then ValveCtrl(12)=0
If IfTime (482,1440,Min) Then ValveCtrl(13)=0
If IfTime (482,1440,Min) Then ValveCtrl(14)=0
If IfTime (482,1440,Min) Then ValveCtrl(15)=0
If IfTime (482,1440,Min) Then ValveCtrl(16)=0
If IfTime (482,1440,Min) Then ValveCtrl(17)=0
If IfTime (482,1440,Min) Then ValveCtrl(18)=0

If IfTime (484,1440,Min) Then ValveCtrl(1)=0
If IfTime (484,1440,Min) Then ValveCtrl(2)=0
If IfTime (484,1440,Min) Then ValveCtrl(3)=0
If IfTime (484,1440,Min) Then ValveCtrl(4)=0
If IfTime (484,1440,Min) Then ValveCtrl(5)=0
If IfTime (484,1440,Min) Then ValveCtrl(6)=0
If IfTime (484,1440,Min) Then ValveCtrl(7)=0
If IfTime (484,1440,Min) Then ValveCtrl(8)=0
If IfTime (484,1440,Min) Then ValveCtrl(9)=0

If IfTime (484,1440,Min) Then ValveCtrl(10)=0
If IfTime (484,1440,Min) Then ValveCtrl(11)=0
If IfTime (484,1440,Min) Then ValveCtrl(12)=0
If IfTime (484,1440,Min) Then ValveCtrl(13)=0
If IfTime (484,1440,Min) Then ValveCtrl(14)=0
If IfTime (484,1440,Min) Then ValveCtrl(15)=0
If IfTime (484,1440,Min) Then ValveCtrl(16)=0
If IfTime (484,1440,Min) Then ValveCtrl(17)=0
If IfTime (484,1440,Min) Then ValveCtrl(18)=0

If IfTime (1265,1440, Min) Then ValveCtrl(19)=0
If IfTime (1265,1440, Min) Then ValveCtrl(20)=0
If IfTime (1265,1440, Min) Then ValveCtrl(21)=0
If IfTime (1265,1440, Min) Then ValveCtrl(22)=0
If IfTime (1265,1440, Min) Then ValveCtrl(23)=0
If IfTime (1265,1440, Min) Then ValveCtrl(24)=0
If IfTime (1265,1440, Min) Then ValveCtrl(25)=0
If IfTime (1265,1440, Min) Then ValveCtrl(26)=0
If IfTime (1265,1440, Min) Then ValveCtrl(27)=0
If IfTime (1265,1440, Min) Then ValveCtrl(28)=0
If IfTime (1265,1440, Min) Then ValveCtrl(29)=0
If IfTime (1265,1440, Min) Then ValveCtrl(30)=0
If IfTime (1265,1440, Min) Then ValveCtrl(31)=0
If IfTime (1265,1440, Min) Then ValveCtrl(32)=0
If IfTime (1265,1440, Min) Then ValveCtrl(33)=0
If IfTime (1265,1440, Min) Then ValveCtrl(34)=0
If IfTime (1265,1440, Min) Then ValveCtrl(35)=0
If IfTime (1265,1440, Min) Then ValveCtrl(36)=0

If IfTime (1265,1440, Min) Then ValveCtrl(48)=0
If IfTime (1262,1440, Min) Then ValveCtrl(1)=0
If IfTime (1262,1440,Min) Then ValveCtrl(2)=0
If IfTime (1262,1440,Min) Then ValveCtrl(3)=0
If IfTime (1262,1440,Min) Then ValveCtrl(4)=0
If IfTime (1262,1440,Min) Then ValveCtrl(5)=0
If IfTime (1262,1440,Min) Then ValveCtrl(6)=0
If IfTime (1262,1440,Min) Then ValveCtrl(7)=0
If IfTime (1262,1440,Min) Then ValveCtrl(8)=0
If IfTime (1262,1440,Min) Then ValveCtrl(9)=0
If IfTime (1262,1440,Min) Then ValveCtrl(10)=0
If IfTime (1262,1440,Min) Then ValveCtrl(11)=0
If IfTime (1262,1440,Min) Then ValveCtrl(12)=0
If IfTime (1262,1440,Min) Then ValveCtrl(13)=0
If IfTime (1262,1440,Min) Then ValveCtrl(14)=0
If IfTime (1262,1440,Min) Then ValveCtrl(15)=0
If IfTime (1262,1440,Min) Then ValveCtrl(16)=0

If IfTime (1262,1440,Min) Then ValveCtrl(17)=0
If IfTime (1262,1440,Min) Then ValveCtrl(18)=0

If IfTime (902,1440, Min) Then ValveCtrl(48)=0
If IfTime (902,1440, Min) Then ValveCtrl(1)=0
If IfTime (902,1440,Min) Then ValveCtrl(2)=0
If IfTime (902,1440,Min) Then ValveCtrl(3)=0
If IfTime (902,1440,Min) Then ValveCtrl(4)=0
If IfTime (902,1440,Min) Then ValveCtrl(5)=0
If IfTime (902,1440,Min) Then ValveCtrl(6)=0
If IfTime (902,1440,Min) Then ValveCtrl(7)=0
If IfTime (902,1440,Min) Then ValveCtrl(8)=0
If IfTime (902,1440,Min) Then ValveCtrl(9)=0
If IfTime (902,1440,Min) Then ValveCtrl(10)=0
If IfTime (902,1440,Min) Then ValveCtrl(11)=0
If IfTime (902,1440,Min) Then ValveCtrl(12)=0
If IfTime (902,1440,Min) Then ValveCtrl(13)=0
If IfTime (902,1440,Min) Then ValveCtrl(14)=0
If IfTime (902,1440,Min) Then ValveCtrl(15)=0
If IfTime (902,1440,Min) Then ValveCtrl(16)=0
If IfTime (902,1440,Min) Then ValveCtrl(17)=0
If IfTime (902,1440,Min) Then ValveCtrl(18)=0

'Watered Tubes- These lines should never be changed'

If IfTime(480,1440,Min) AND VWC_5<0.38 AND VWC_5>0.1 Then ValveCtrl(19)=1
If IfTime(480,1440,Min) AND VWC_13<0.38 AND VWC_13>0.1 Then ValveCtrl(20)=1
If IfTime(480,1440,Min) AND VWC_21<0.38 AND VWC_21>0.1 Then ValveCtrl(21)=1
If IfTime(480,1440,Min) AND VWC_29<0.38 AND VWC_29>0.1 Then ValveCtrl(22)=1
If IfTime(480,1440,Min) AND VWC_37<0.38 AND VWC_37>0.1 Then ValveCtrl(23)=1
If IfTime(480,1440,Min) AND VWC_45<0.38 AND VWC_45>0.1 Then ValveCtrl(24)=1
If IfTime(480,1440,Min) AND VWC_53<0.38 AND VWC_53>0.1 Then ValveCtrl(25)=1
If IfTime(480,1440,Min) AND VWC_61<0.38 AND VWC_61>0.1 Then ValveCtrl(26)=1
If IfTime(480,1440,Min) AND VWC_69<0.38 AND VWC_69>0.1 Then ValveCtrl(27)=1
If IfTime(480,1440,Min) AND VWC_77<0.38 AND VWC_77>0.1 Then ValveCtrl(28)=1
If IfTime(480,1440,Min) AND VWC_85<0.38 AND VWC_85>0.1 Then ValveCtrl(29)=1
If IfTime(480,1440,Min) AND VWC_93<0.38 AND VWC_93>0.1 Then ValveCtrl(30)=1
If IfTime(480,1440,Min) AND VWC_101<0.38 AND VWC_101>0.1 Then ValveCtrl(31)=1
If IfTime(480,1440,Min) AND VWC_109<0.38 AND VWC_109>0.1 Then ValveCtrl(32)=1
If IfTime(480,1440,Min) AND VWC_117<0.38 AND VWC_117>0.1 Then ValveCtrl(33)=1
If IfTime(480,1440,Min) AND VWC_125<0.38 AND VWC_125>0.1 Then ValveCtrl(34)=1
If IfTime(480,1440,Min) AND VWC_133<0.38 AND VWC_133>0.1 Then ValveCtrl(35)=1
If IfTime(480,1440,Min) AND VWC_141<0.38 AND VWC_141>0.1 Then ValveCtrl(36)=1

If IfTime (485,1440, Min) Then ValveCtrl(1)=0
If IfTime (485,1440,Min) Then ValveCtrl(2)=0

If IfTime (485,1440,Min) Then ValveCtrl(3)=0
 If IfTime (485,1440,Min) Then ValveCtrl(4)=0
 If IfTime (485,1440,Min) Then ValveCtrl(5)=0
 If IfTime (485,1440,Min) Then ValveCtrl(6)=0
 If IfTime (485,1440,Min) Then ValveCtrl(7)=0
 If IfTime (485,1440,Min) Then ValveCtrl(8)=0
 If IfTime (485,1440,Min) Then ValveCtrl(9)=0
 If IfTime (485,1440,Min) Then ValveCtrl(10)=0
 If IfTime (485,1440,Min) Then ValveCtrl(11)=0
 If IfTime (485,1440,Min) Then ValveCtrl(12)=0
 If IfTime (485,1440,Min) Then ValveCtrl(13)=0
 If IfTime (485,1440,Min) Then ValveCtrl(14)=0
 If IfTime (485,1440,Min) Then ValveCtrl(15)=0
 If IfTime (485,1440,Min) Then ValveCtrl(16)=0
 If IfTime (485,1440,Min) Then ValveCtrl(17)=0
 If IfTime (485,1440,Min) Then ValveCtrl(18)=0
 If IfTime (488,1440,Min) Then ValveCtrl(19)=0
 If IfTime (488,1440,Min) Then ValveCtrl(20)=0
 If IfTime (488,1440,Min) Then ValveCtrl(21)=0
 If IfTime (488,1440,Min) Then ValveCtrl(22)=0
 If IfTime (488,1440,Min) Then ValveCtrl(23)=0
 If IfTime (488,1440,Min) Then ValveCtrl(24)=0
 If IfTime (488,1440,Min) Then ValveCtrl(25)=0
 If IfTime (488,1440,Min) Then ValveCtrl(26)=0
 If IfTime (488,1440,Min) Then ValveCtrl(27)=0
 If IfTime (488,1440,Min) Then ValveCtrl(28)=0
 If IfTime (488,1440,Min) Then ValveCtrl(29)=0
 If IfTime (488,1440,Min) Then ValveCtrl(30)=0
 If IfTime (488,1440,Min) Then ValveCtrl(31)=0
 If IfTime (488,1440,Min) Then ValveCtrl(32)=0
 If IfTime (488,1440,Min) Then ValveCtrl(33)=0
 If IfTime (488,1440,Min) Then ValveCtrl(34)=0
 If IfTime (488,1440,Min) Then ValveCtrl(35)=0
 If IfTime (488,1440,Min) Then ValveCtrl(36)=0
 If IfTime (488,1440,Min) Then ValveCtrl(48)=0 'turn fertilizer tank pump off

SDMCD16AC (ValveCtrl(), 3,0)

$T_kPa_33 = (FullBR_3(1) * 79.35 + 56.02) / 10$
 $T_kPa_34 = (FullBR_3(2) * 79.35 + 56.02) / 10$
 $T_kPa_35 = (FullBR_3(3) * 79.35 + 56.02) / 10$
 $T_kPa_36 = (FullBR_3(4) * 79.35 + 56.02) / 10$
 $T_kPa_37 = (FullBR_3(5) * 79.35 + 56.02) / 10$
 $T_kPa_38 = (FullBR_3(6) * 79.35 + 56.02) / 10$
 $T_kPa_39 = (FullBR_3(7) * 79.35 + 56.02) / 10$
 $T_kPa_40 = (FullBR_3(8) * 79.35 + 56.02) / 10$

T_kPa_41=(FullBR_3(9)*79.35+56.02)/10
T_kPa_42=(FullBR_3(10)*79.35+56.02)/10
T_kPa_43=(FullBR_3(11)*79.35+56.02)/10
T_kPa_44=(FullBR_3(12)*79.35+56.02)/10
T_kPa_45=(FullBR_3(13)*79.35+56.02)/10
T_kPa_46=(FullBR_3(14)*79.35+56.02)/10
T_kPa_47=(FullBr_3(15)*79.35+56.02)/10
T_kPa_48=(FullBR_3(16)*79.35+56.02)/10
T_kPa_49=(FullBR(1)*79.35+56.02)/10
T_kPa_50=(FullBR(2)*79.35+56.02)/10
T_kPa_51=(FullBR(3)*79.35+56.02)/10
T_kPa_52=(FullBR(4)*79.35+56.02)/10
T_kPa_53=(FullBR(5)*79.35+56.02)/10
T_kPa_54=(FullBR(6)*79.35+56.02)/10

'Call Data Tables and Store Data
CallTable Tens2
CallTable Table2
CallTable WateringRecord
NextScan