MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 75-2015 (YEAR 2)
2015 Annual Report

Project title: Soybean Physiological Maturity: Documentation and Developing a Tool for Management

Name of the PI and email address: K. Raja Reddy; E-mail: krreddy@pss.msstate.edu

REPORT OF PROGRESS/ACTIVITY

Objectives: The primary objective of this project is to precisely identify and quantify the effects of a wide range of soil moisture stress levels on growth, and developmental, and physiological performances of two soybean cultivars through a series of programmed evapotranspiration (ET)-based irrigation experiments utilizing sunlit plant growth chambers.

To fulfill our objectives, three controlled environment experiments were conducted using two varieties of soybean, indeterminate Asgrow 5232 and determinate Progeny 5333. In all experiments, five drought stress treatments (100, 80, 60, 40, and 20% ET) were imposed to study soybean responses to different soil moisture stress levels at each specific growth stage.

Experiment 1: Root architecture and vegetative growth responses of soybean to early season soil moisture deficit.

To understand root architecture and seedling vigor, two experiments were conducted. In the first study, the treatments were imposed immediately after seed emergence, and root growth and early-seedling growth were studied at 18 days after sowing. In the second study, five soil moisture stress treatments were imposed after seedling emergence or 7 days after planting (DAP), and plant growth and development including root traits were measured at 29 DAP.

In both the studies, the experiments were arranged as a completely randomized design with nine replicates. Plants were fertigated using full-strength Hoagland nutrient solution, and five levels of soil moisture treatments [100%, 80%, 60%, 40%, and 20% of evapotranspiration (ET)] were imposed accordingly. Continuous monitoring of soil moisture content was conducted using soil moisture sensors inserted at a depth of 10 cm in every five random pots of each soil moisture treatment.

Gas exchange measurements were taken on the youngest, fully expanded leaf from three individual plants per treatment between 1100 and 1300 h using LI-6400 photosynthesis system (LI-COR, Inc.) fitted with an integrated fluorescence chamber head (LI-6400 leaf chamber fluorometer; LI-COR, Inc.).

At the final harvest, leaf area, number of nodes, and all plant component dry weights were measured. Root images were taken using winRHIZO root image analysis system and images were analyzed to understand the early season root growth.

Preliminary Results

To our knowledge, this is the first study to identify root growth and root architecture response to a wide range of soil moisture regimes that could be beneficial for management decisions in the field. Observations of the root systems of the two cultivars (Asgrow 5332 and Progeny 5333) in both the studies at18 and 29 DAP revealed differences that were visually distinct based on the water stress treatments (Fig. 2 and 7). Asgrow 5332 had a larger, well-structured root system with higher values for root traits, while Progeny 5333 exhibited a comparatively less-structured and poorly organized root system.

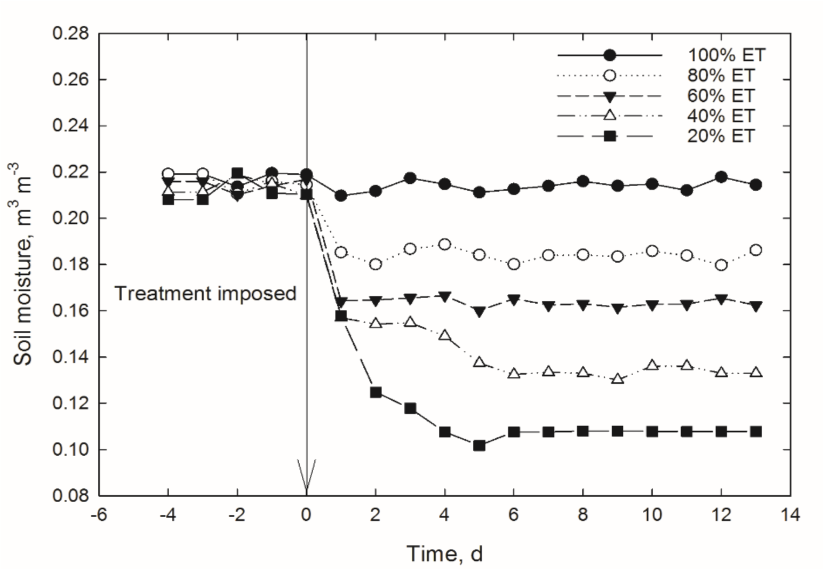


Fig. 1. *Volumetric soil moisture content across the treatments before and during the experimental period which were maintained using sensor-based monitoring and evapotranspiration based irrigation system. The arrow indicates the day in which the treatments were imposed.*

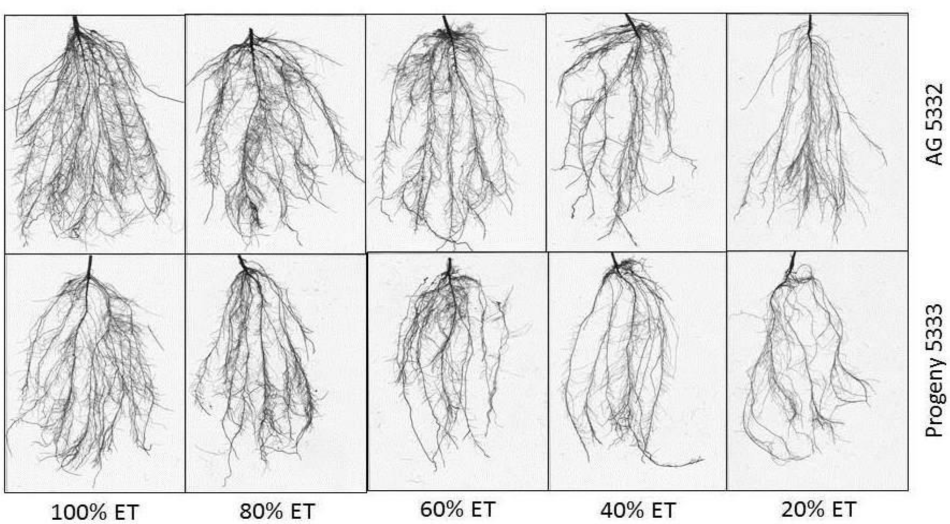


Fig. 2. *Representative scanned root images for two soy bean cultivars from five water stress treatments harvested at 18 days after planting (DAP). Treatments were imposed 4 DAP.*

Plant height (Fig. 3A) and node no. (Fig. 3B) showed a significant and quadratic decline with increasing soil moisture levels in both cultivars, whereas leaf area (Fig. 3B) decreased linearly with increasing water stress without showing a significant difference between cultivars.

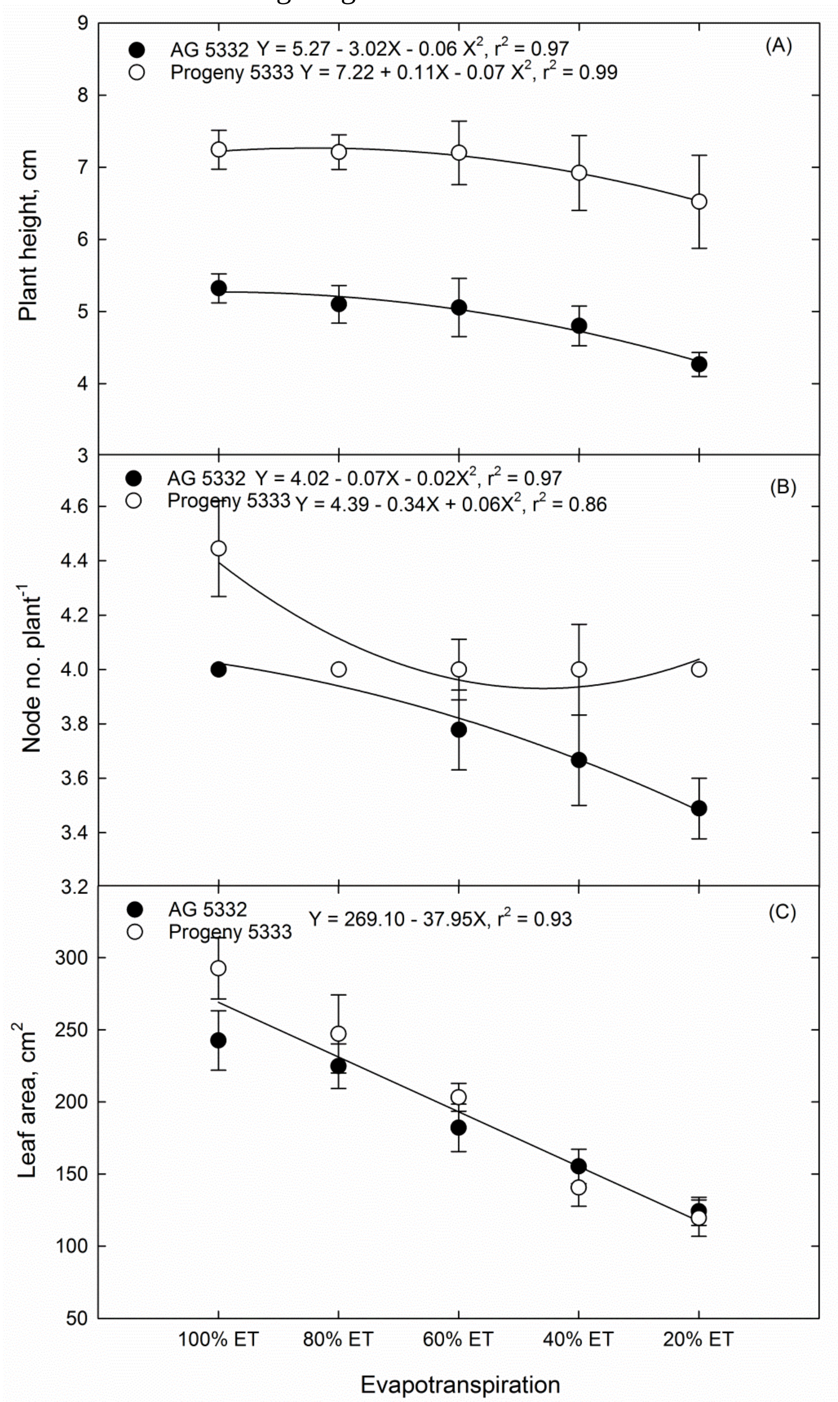


Fig. 3. *Influence of soil moisture content on (A) plant height, (B) Node no., and (C) leaf area harvested at 18 d after planting for two soybean cultivars. Solid lines are predicted values of the respective fitted equations and the error bars indicate the standard error of the means (n = 9).*

Progeny 5333 had the greatest vegetative growth when compared to Asgrow 5332. On the other hand, Asgrow 5332 exhibited higher physiological performance (Fig. 4) and root growth (Fig. 5) than Progeny 5333 under different moisture stress treatments.

Net photosynthesis (Fig. 4A) and stomatal conductance (Fig. 4B) decreased linearly for AG 5332 with increasing water stress, while the decline was quadratic for Progeny 5333. The significant differences between the two cultivars were observed for net photosynthesis and stomatal conductance. However, transpiration (Fig. 4C) difference was not significant between the cultivars, and it decreased linearly with increasing moisture stress.

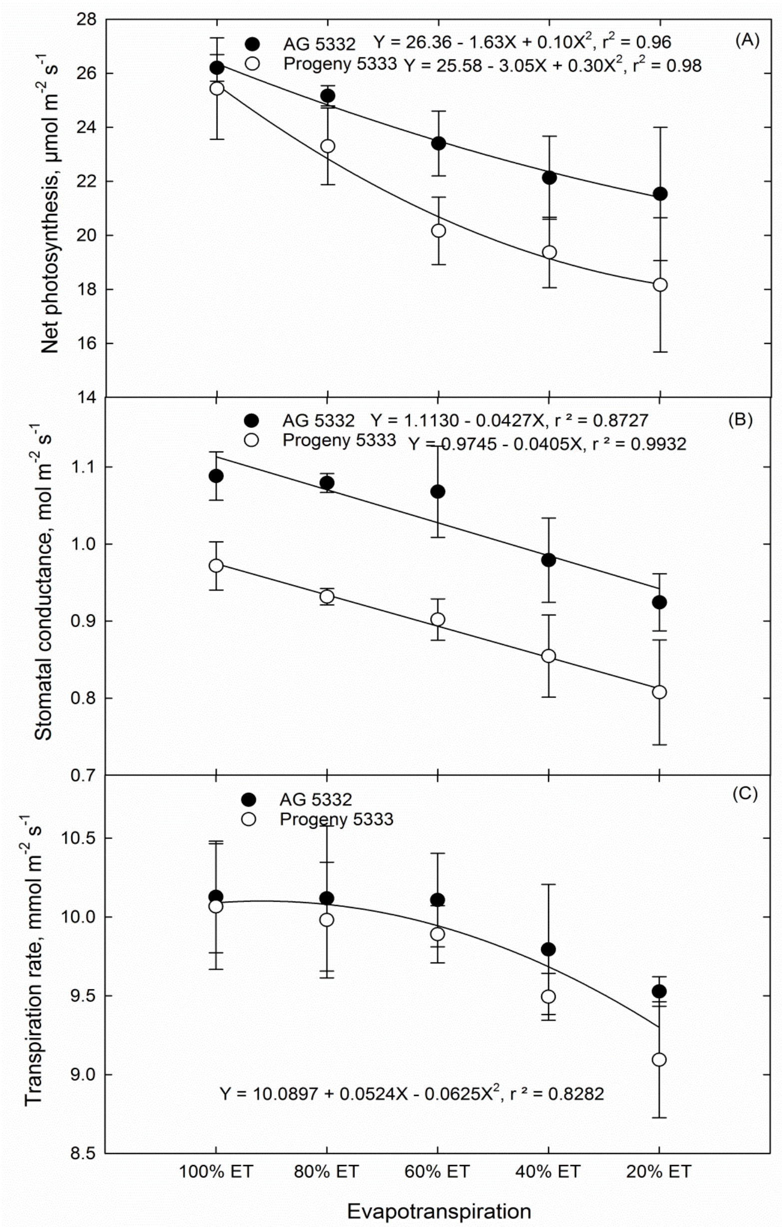


Fig. 4. *Influence on soil moisture content on (A) net photosynthesis, (B) stomatal conductance, and (C) transpiration rate measured at 16 d after planting for two soy bean cultivars. Solid lines are predicted values of the respective fitted equations and the error bars indicate the standard error of the means (n = 9).*

Root growth responded in a similar way to shoot growth, with a decline in root length (Fig. 5A), root surface area (Fig. 5B), and root diameter (Fig. 5C) with increasing the moisture stress. Root length and root surface area showed significant differences between the cultivars, whereas root diameter exhibited a quadratic response that was not significantly different between cultivars.

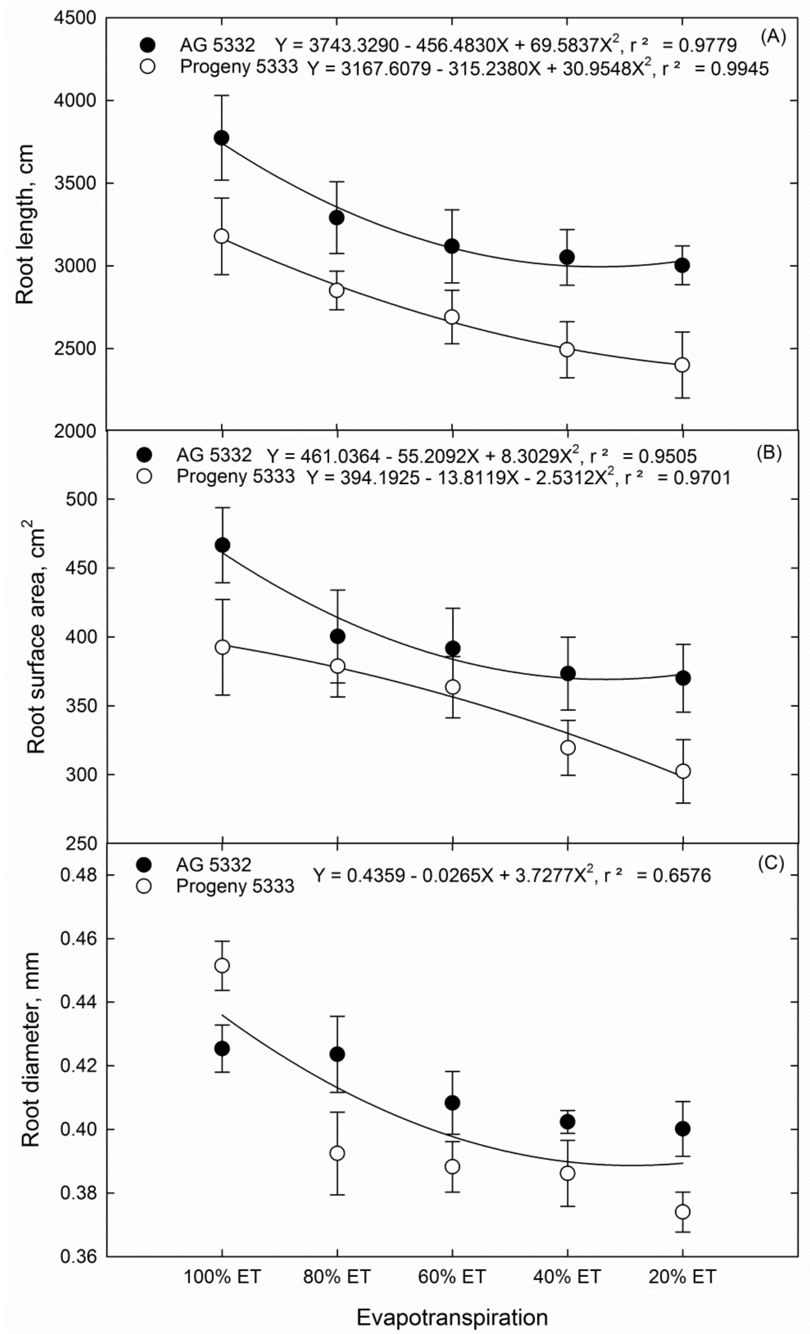


Fig. 5. *Influence on soil moisture content on (A) root length, (B) root surface area, and (C) root diameter harvested at 18 d after planting for two soy bean cultivars. Solid lines are predicted values of the respective fitted equations and the error bars indicate the standard error of the means (n = 9).*

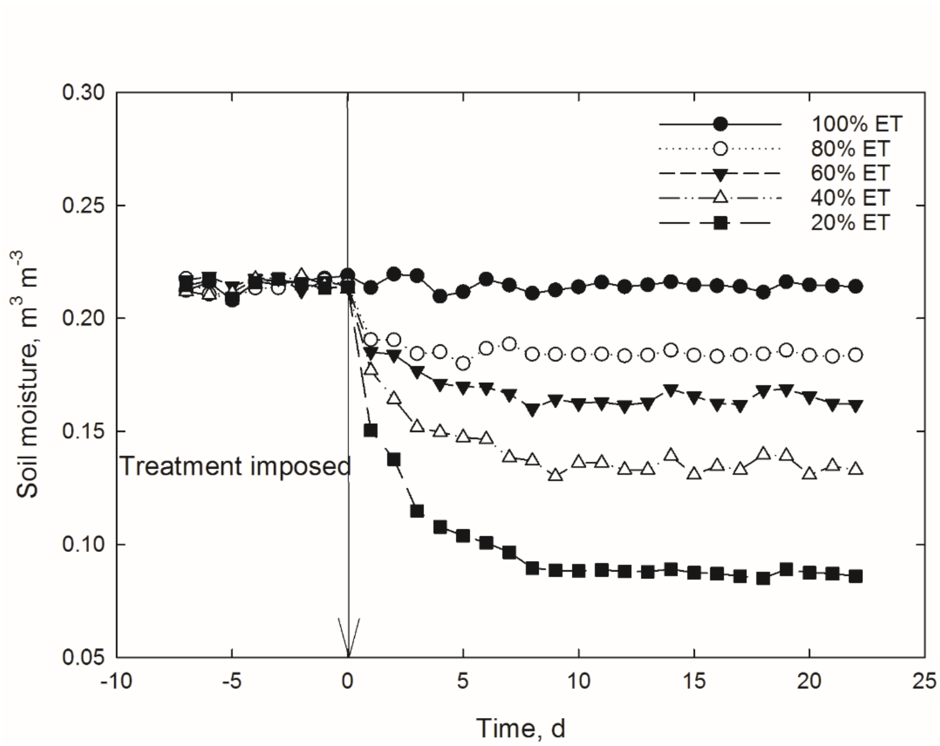


Fig. 6. *Volumetric soil moisture content across the treatments before and during the experimental period which were maintained using sensor-based monitoring and evapotranspiration based irrigation system. The arrow indicates the day in which the treatments were imposed.*

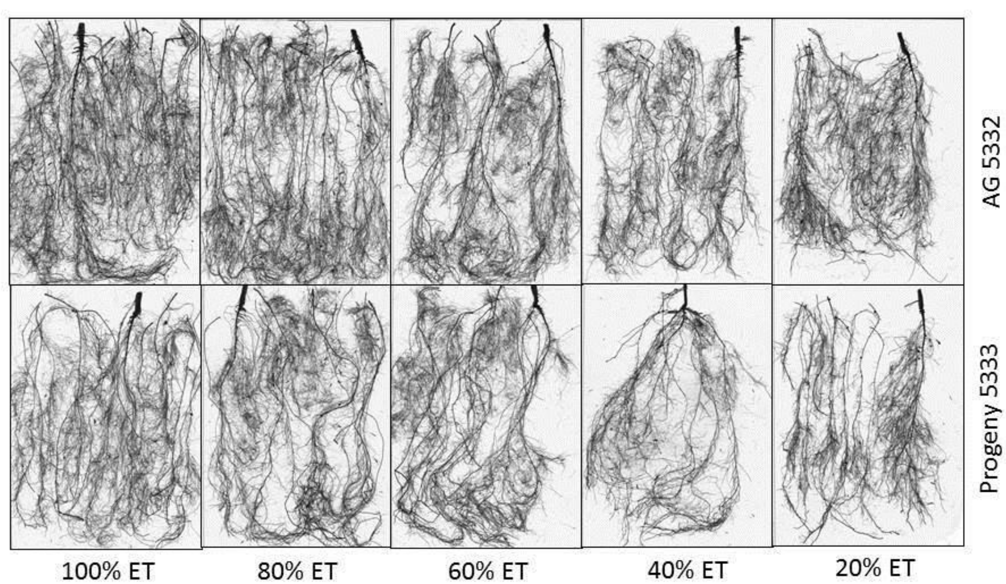


Fig. 7. *Representative scanned root images for two soybean cultivars from five water stress treatments harvested at 29 d after planting. Treatments were imposed 7 DAP.*

Table 1 represents the mean values of all morphological and physiological parameters measured for two soybean cultivars at 29 DAP. Morphological parameters include vegetative growth traits such as plant height, leaf no., leaf area, shoot and root component dry weights and seven different root traits.

Experiment 2: Soil moisture deficit effects on soybean vegetative growth and development

The objectives of this study were to determine soil moisture stress effects on vegetative (V Stage) development and growth and physiological processes up to R1 stage. Pots were arranged as a completely randomized design in 6 rows with 3 pots per row in each SPAR chamber, with 9 pots for each cultivar. All SPAR chambers were maintained at 400 µmol mol^{-1} [CO_2] and 29/21°C day/night temperature until harvest.

Five water stress treatments, 100, 80, 60, 40, and 20% of daily ET of the control, were imposed at 31 DAP and were continued until 65 DAP. Moisture content was monitored continuously until the end of the experiment. During the experimental period, leaves on the main stem were measured on nine plants in each treatment of both cultivars.

Midday leaf water potential (LWP) was measured with a Scholander Pressure Chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). Measurements were taken four times during the growth period (46, 52, 57, and 62 DAP, between 1200 and1300 h) from the most recent fully expanded leaves (fourth or fifth leaf from the top), and from three plants per cultivar. Gas exchange measurements were made using LI-6400 photosynthesis system (LI-COR, Inc.) Leaf photosynthetic pigment content (chlorophyll a, chlorophyll b, and carotenoids), waxes, and cell membrane thermostability (CMT) were measured by taking two sets of leaf samples collected from the five most recent fully expanded leaves from each cultivar and treatment at 60 DAP.

Preliminary Results

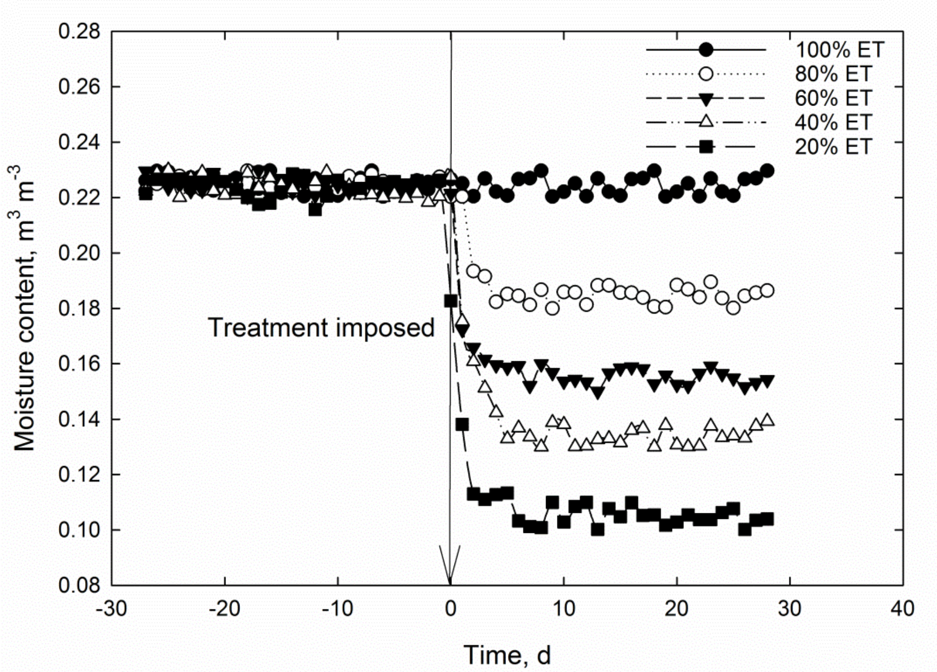
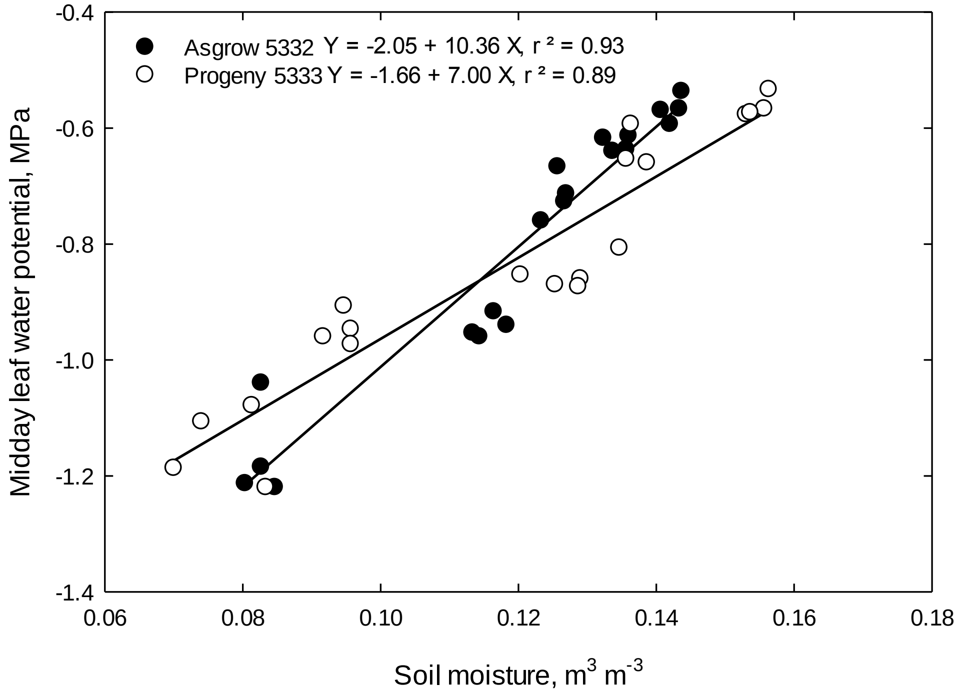


Fig. 8. *Volumetric soil moisture content across the treatments before and during the experimental period which were maintained using sensor-based monitoring and evapotranspiration based irrigation system. The arrow indicates the day in which the treatments were imposed.*

The mean values for the vegetative and physiological traits of two soybean cultivars at R3 stage under each of the soil moisture stress level are given in Table 2.



2.

Fig. 9. *Relationship between soil moisture content and midday leaf water potential (LWP) of two soybean cultivars.*

The midday LWP measured at 46, 52, 57, and 62 DAP ranged between -0.53 and -1.22 MPa, and increased linearly with increasing soil moisture content (Fig. 9). A significant difference was observed between cultivars for midday LWP.

Similar to midday LWP, net photosynthesis also increased linearly with increasing soil moisture levels (Fig. 10). The rate of net photosynthesis of Asgrow 5332 was significantly higher than that of Progeny 5333. However, this difference between cultivars did not exist towards the lower soil moisture conditions. Averaged across cultivars, net photosynthesis rate dropped by 40% from the highest to lowest soil moisture measured in this study.

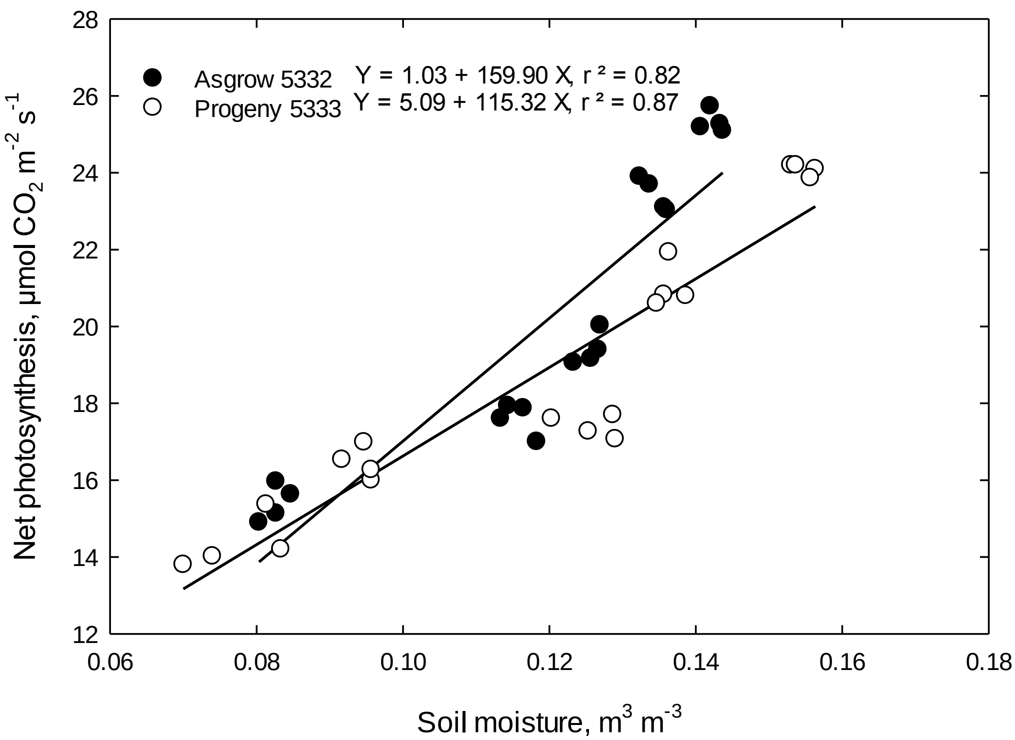


Fig. 10. *Relationship between soil moisture content and net photosynthesis of two soybean cultivars.*

Experiment 3: Soil moisture deficit effects on soybean reproductive growth, development, seed yield, and phenotypic evaluation of yield distribution

The objectives of this study were to quantify the effects of different levels of soil moisture on soybean reproductive growth, R-state development, seed quality, and yield to develop functional relationships for crop modeling. Initially, pots were arranged outdoors until the plants reached R1 stage. Afterwards, pots were moved into SPAR chambers. Five SPAR units were used for each cultivar.

Five water stress treatments, 100, 80, 60, 40, and 20% of daily ET of the control, were imposed at 41 DAP and continued until harvest. Reproductive growth and development were recorded. Measurements of photosynthesis and mid-day leaf water potential were taken at 61, 65, 68, 70 and 72 DAP. Leaf photosynthetic pigment content (chlorophyll a, chlorophyll b, and carotenoids), waxes, and cell membrane thermostability (CMT) were measured two times, 62 DAP and at harvest, by taking two sets of leaf samples collected from the five most recent fully expanded leaves from each cultivar and treatment. Seed quality analysis will be carried out at the USDA-ARS Crop Genetics Research Unit, Stoneville MS. Seed from each treatment will be analyzed for protein, oil, fatty acids, sucrose, raffinose, and stachyose.

Preliminary Results

Fig. 11. *Pictorial representation of soybean Asgrow 5332 cultivar grown at 100%, 80%, 60%, 40%, and 20% ET in the SPAR chambers to study soil moisture stress effects on soybean growth, yield and yield components.*

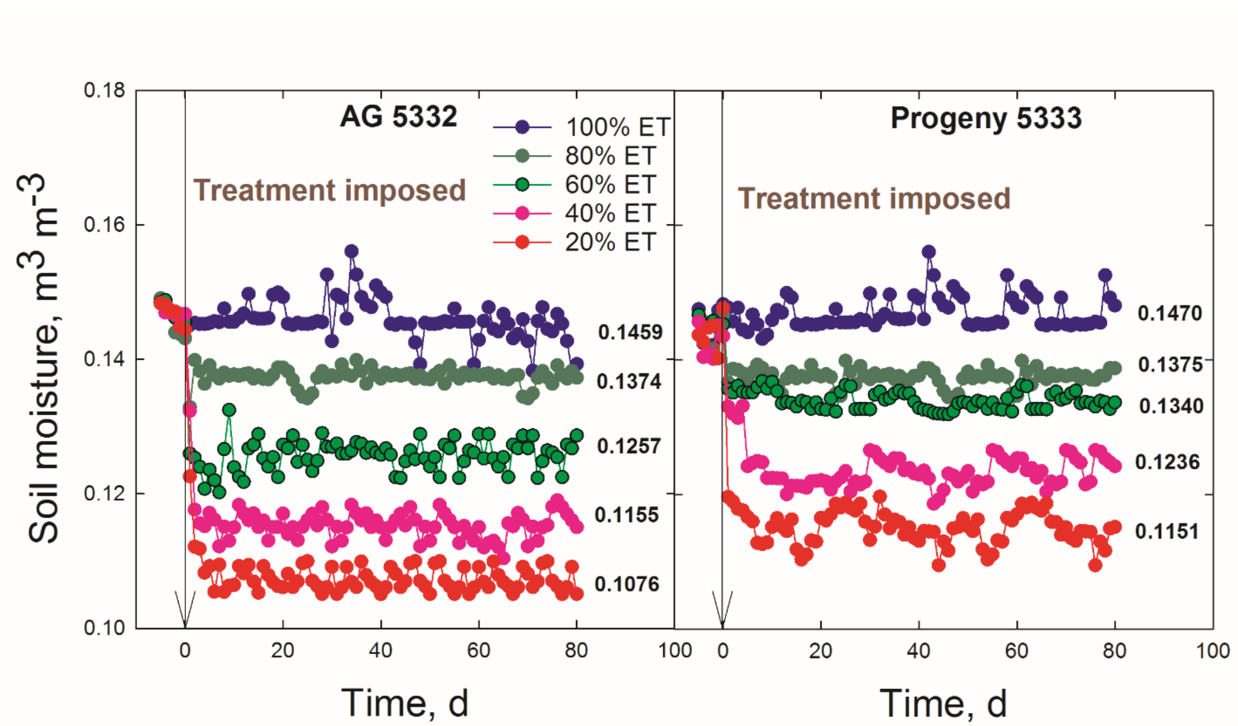


Fig. 12. *Volumetric soil moisture content across the treatments before and during the experimental period which were maintained using sensor-based monitoring and evapotranspiration based irrigation system. The arrow indicates the day in which the treatments were imposed.*

The measured soil moisture content (Fig. 12) with minimum variability during the experimental period from flowering to maturity was accomplished by measuring ET and soil moisture, and adjusting irrigation on a two-day basis based on those values throughout the experiment. Soil moisture levels varied significantly among the different ET-based irrigation treatments throughout the treatment period, and were 0.146 $m^3 m^{-3}$ soil for the 100% ET, 0.137 $m^3 m^{-3}$ soil for the 80% ET, 0.130 $m^3 m^{-3}$ soil for the 60% ET, 0.117 $m^3 m^{-3}$ soil for the 64% ET, and 0.111 $m^3 m^{-3}$ soil for the 20% ET treatments when averaged between the measured values for the each of respective irrigation treatment values.

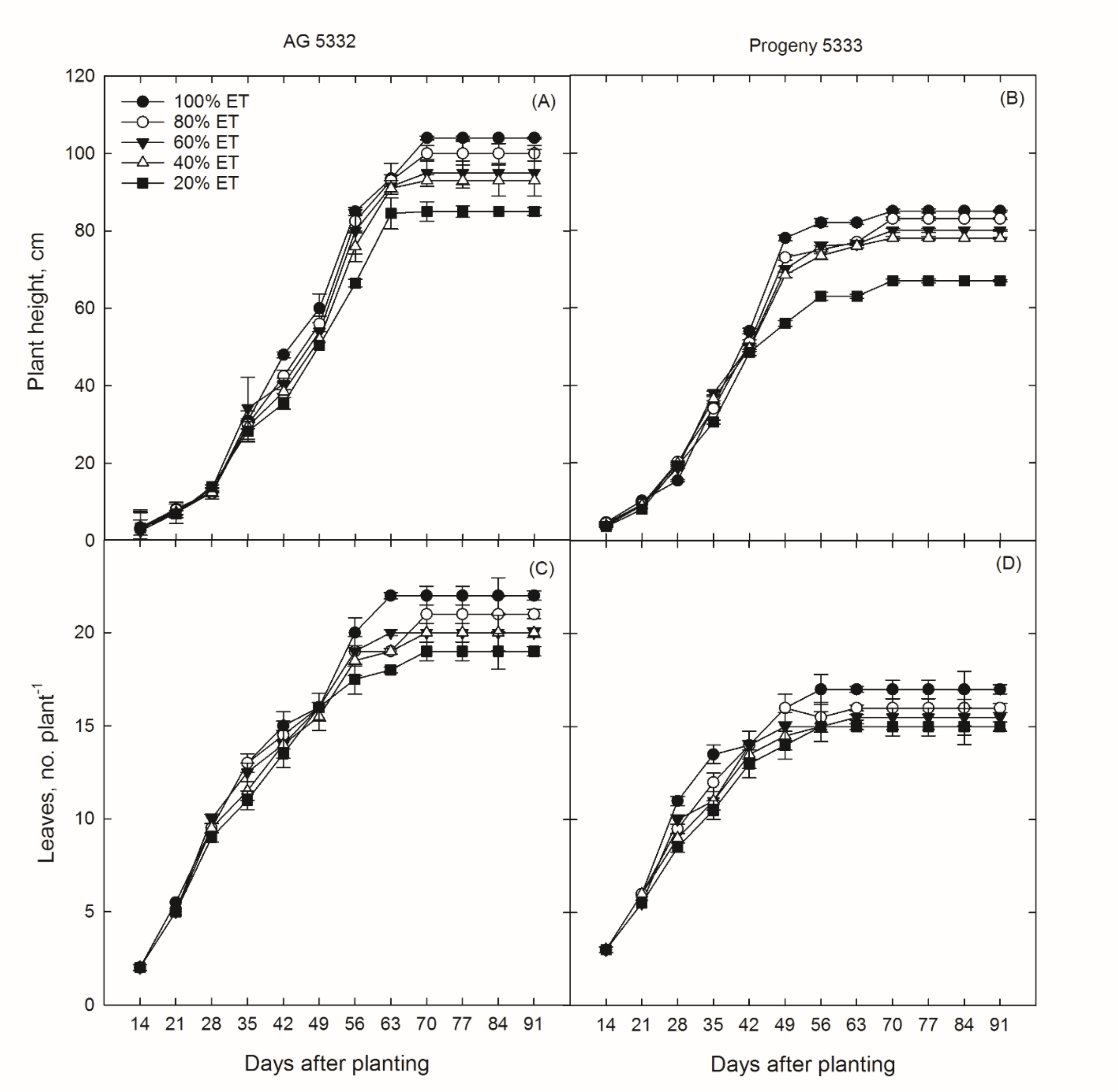


Fig. 13. *Time-series analysis of plant height and nodes in two soybean cultivars, Asgrow 5232 and Progeny 5333, across five soil moisture regimes.*

Soil moisture regimes significantly influenced plant height and number of nodes produced on both cultivars (Fig. 13). Even though plant height and number of leaves increased over time during the treatment period, final plant height was affected by soil moisture regimes. AG 5332 had the tallest plants (Fig. 13A) and greater number of nodes (Fig. 13C) when compared to Progeny 5333 (Fig. 13B and 13D) under each of the soil moisture regimes.

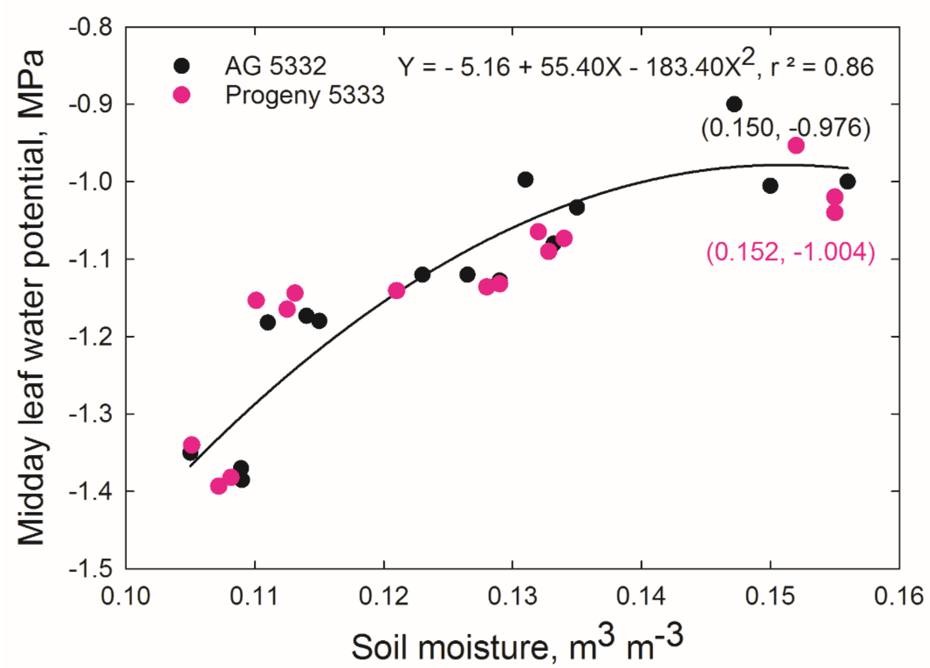


Fig. 14. *Relationship between soil moisture content and midday LWP for two soybean cultivars.*

The relationship between midday leaf water potential (LWP) and soil moisture was quadratic and the difference was not significant between cultivars (Fig. 14). However, the maximum LWP varied between cultivars, where Asgrow 5332 reached its maximum LWP (-0.976 MPa) under 0.150 m^3 m^{-3} soil moisture level, while Progeny 5333 attained its maxima of -1.004 MPa at 0.152 m^3 m^{-3} soil moisture level.

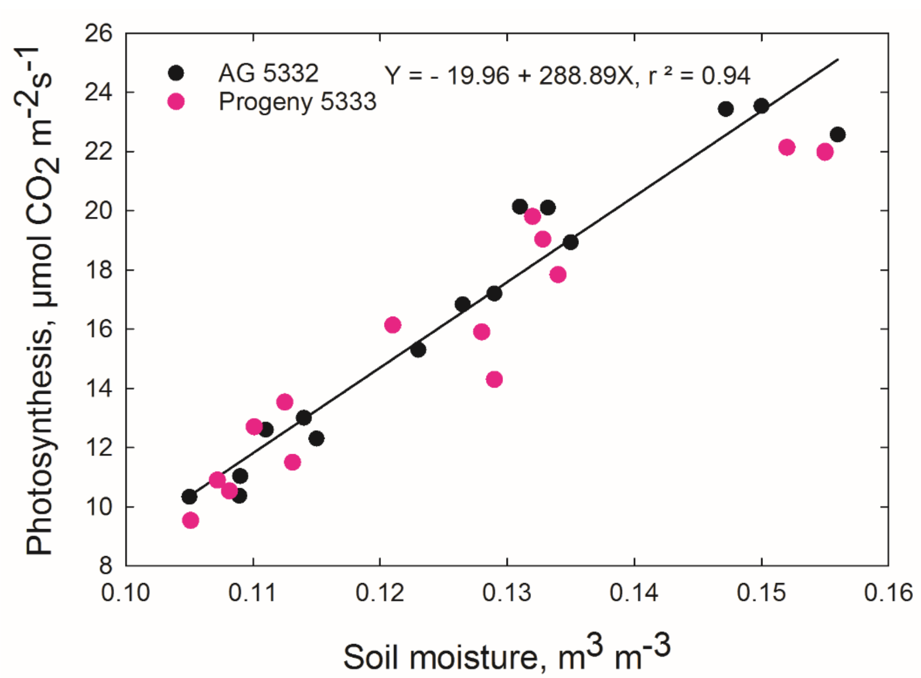


Fig. 15. *Relationship between soil moisture content and net photosynthesis for two soybean cultivars.*

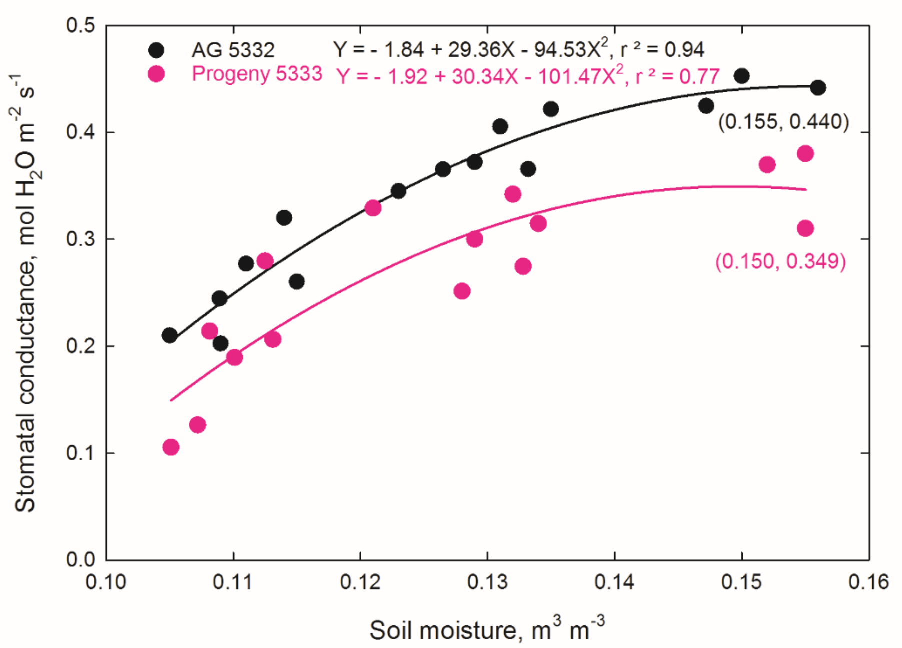


Fig. 16. *Relationship between soil moisture content and stomatal conductance for two soybean cultivars.*

A single linear function best described the relationship between photosynthesis and soil moisture content in both cultivars (Fig. 15). The rate of increase was moles per unit of soil moisture content, where the increase was not significant between cultivars. Maximum leaf photosynthetic rate (23.5 μmol m^{-2} s^{-1}) was observed for the control treatment at about 0.15 m^3 m^{-3} soil moisture (100% ET), and the linear decline in photosynthetic rates are being reflected in biomass reduction on various plant components.

Stomatal conductance increased significantly and quadratically with increasing soil moisture content (Fig. 16). The soil moisture optima varied between the cultivars for stomatal conductance.

Soybean component biomass production was significantly ($P \leq 0.05$) affected by soil moisture deficit stress that was regulated by ET-based irrigation treatments (Fig. 17). The leaf, stem, root, and seed dry weights and total biomass declined linearly with decreasing soil moisture levels for both cultivars. Asgrow 5332 had higher biomass production than progeny 5333. The reduction of total biomass was 40% in AG 5332 under most drought stressed condition compared to its control, whereas progeny 5333 showed a 54% reduction.

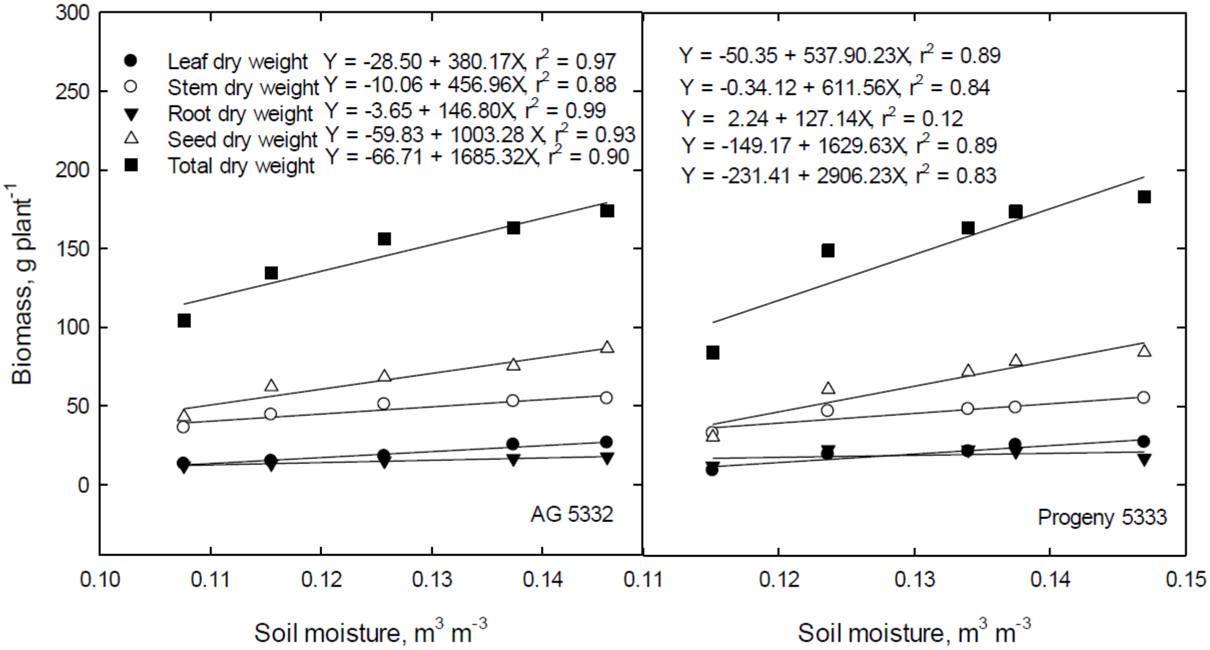


Fig. 17. Relationships between soil moisture and total and component (stem, leaf, root, and seed) biomass production of soybean, cultivars, Asgrow 5332 and progeny 5333, at 125 days after planting. Bars represent standard errors of the mean (n=12) and are shown when larger than the symbols.

The distribution of pods on the mainstem and branches varied considerably in both cultivars (Fig. 18) and among ET-based irrigation treatments. The middle region of the canopy in both cultivars was responsible for approximately 55% of final yield compared to top or bottom regions (Fig. 19). The pods were more evenly distributed for Asgrow 5332 from nodes 4 to 23 under most of the moisture stress regimes, while the pod distribution was limited to node 17 starting from node 5 in Progeny 5333 cultivar (Fig. 19). However, more pods was measured on Progeny 5333 than Asgrow 5332 in the middle canopy of the maintsem. In contrast to main stem pods, Asgrow 5332 had more pods on branches compared to Progeny 5333 (Fig. 20). The first four branches of both cultivars contributed more pods under all moisture stress treatments.

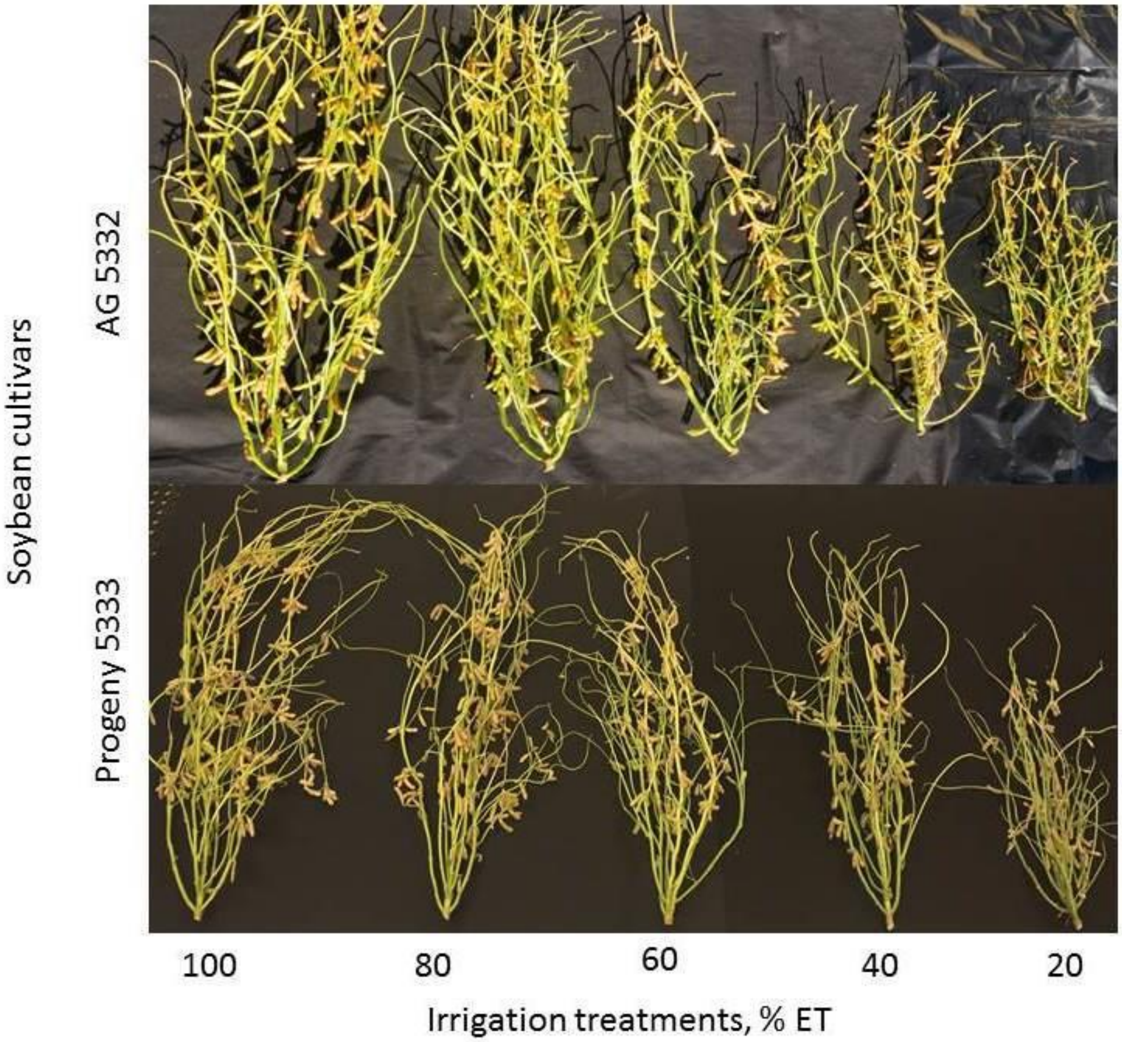


Fig. 18. *Influence of soil moisture content on pod distribution patterns of two soy bean cultivars. Plants were harvested at R8 stage.*

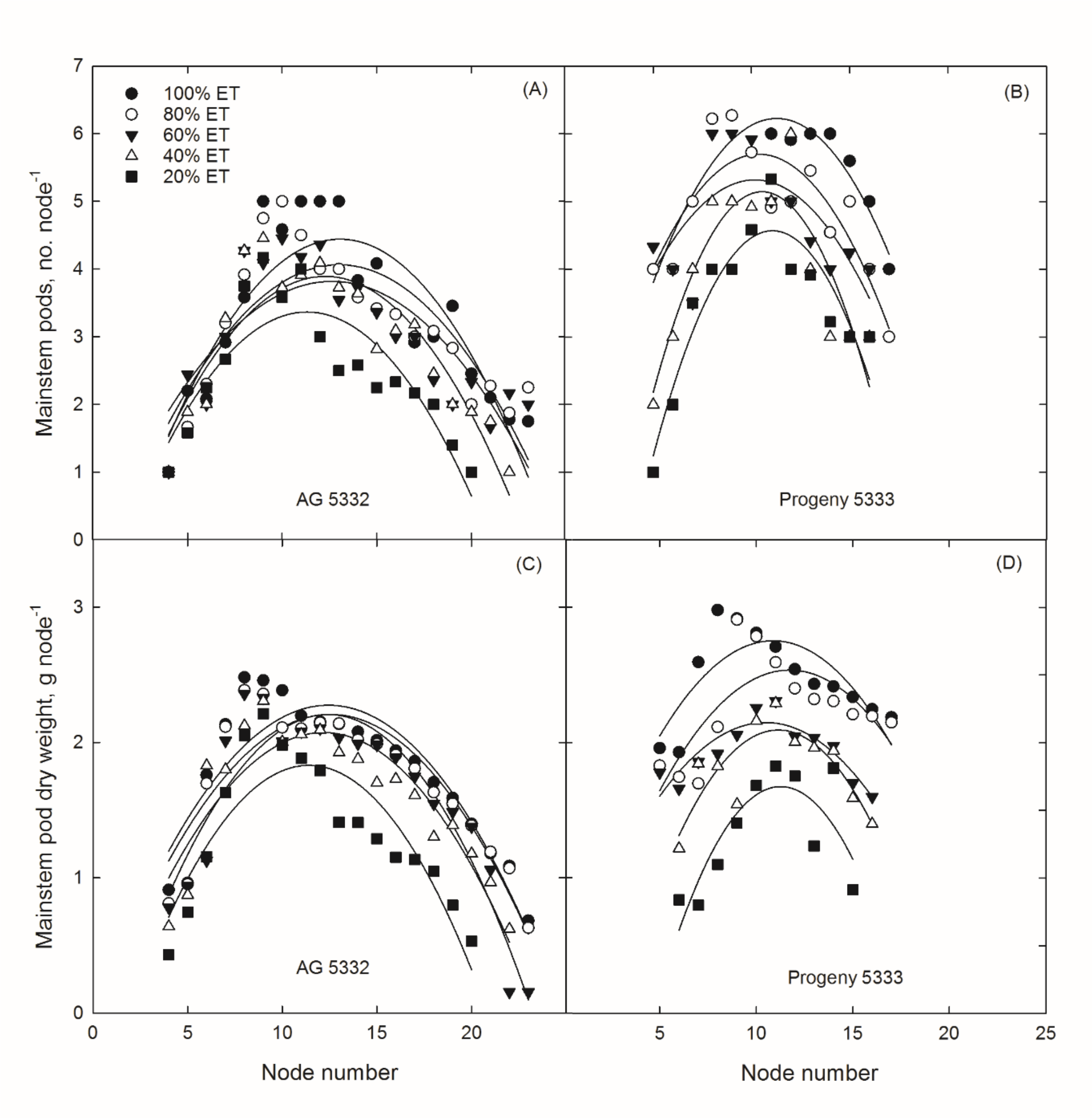


Fig. 19. *Influence of soil moisture content on main stem pod no., and main stem pod dry weight in each canopy region for two soy bean cultivars. Plants were harvested at full maturity.*

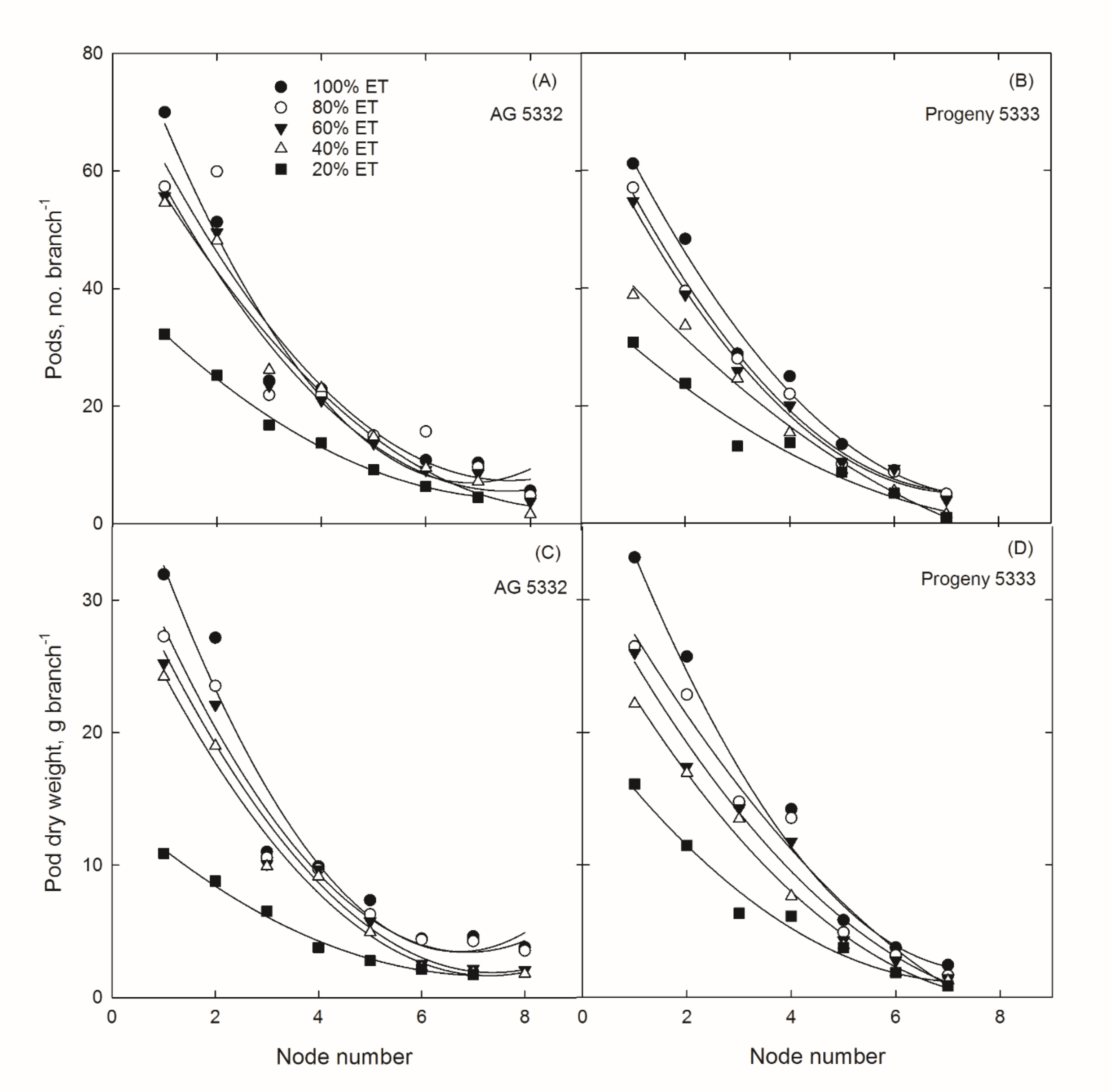


Fig. 20. *Influence of soil moisture content on branch pod no., and branch pod dry weight in each canopy region for two soy bean cultivars. Plants were harvested at full maturity.*

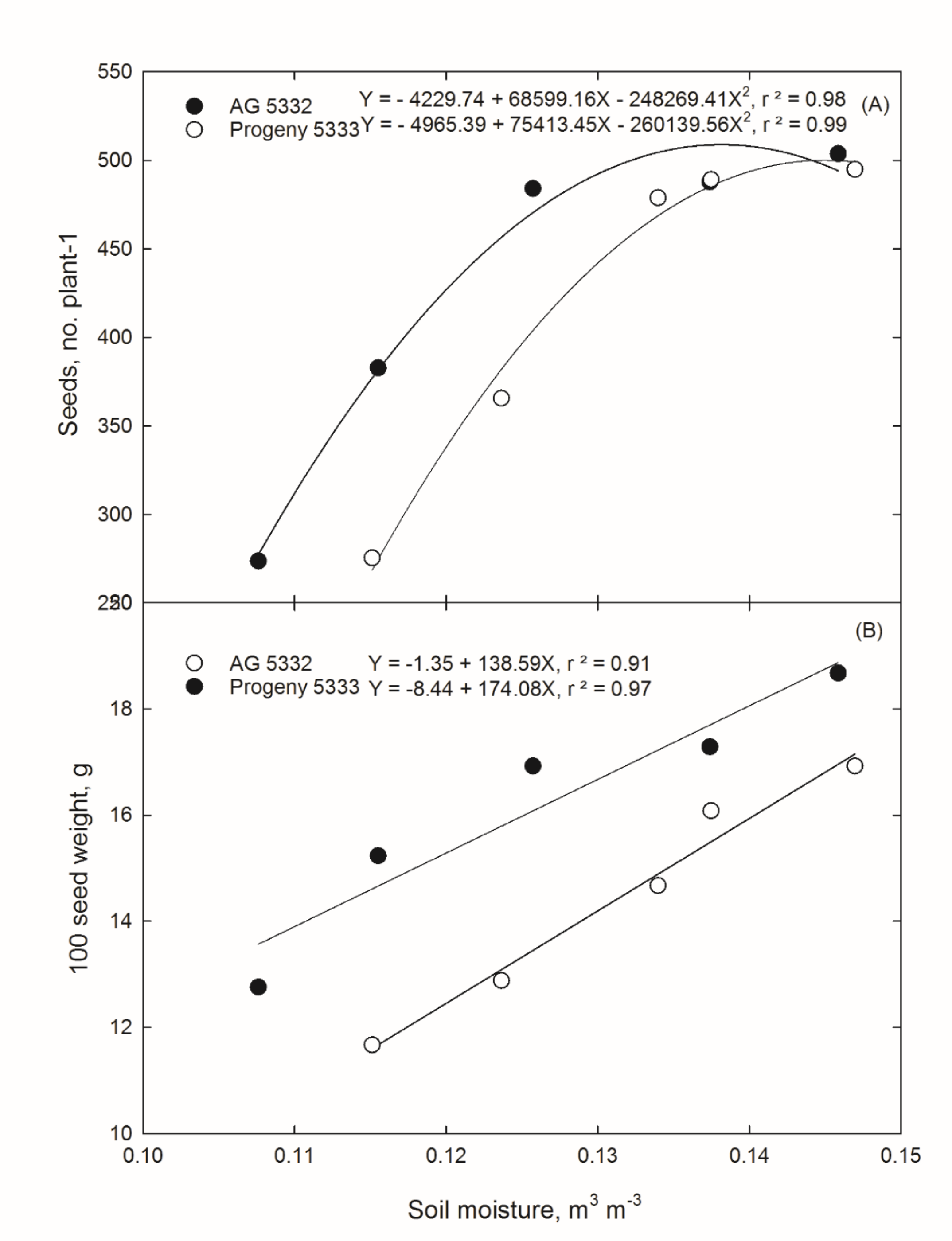


***Fig. 21.** Relationship between soil moisture content vs seed no.; and 100 seed weight. Soil moisture content was measured at the 10- cm depth soil column.*

Approximately 45% and 64% reductions in seed number and seed weight were observed under 20% moisture stress compared to the control for Progeny 5333, while Asgrow 5332 had a 46% and 43% decline for those yield components at the respective treatment levels (Fig.21A). The 100 seed weight was much greater for seed of AG 5332 compared to seed of Progeny 5333 across all soil moisture levels, and the rate of decline was greater for seed of Progeny 5333 than AG in response to decline in soil moisture levels. (Fig.21B).

Total and seed dry weights differed significantly between cultivars (Fig. 22). Soil moisture deficits decreased both total and seed dry weights and the decline was greater in Progeny than in Asgrow 5332. The decline in biomass was attributed to decreased photosynthesis and loss of leaves due to senescence. The three lowest soil moisture stress treatments produced small, shriveled seeds in both cultivars.

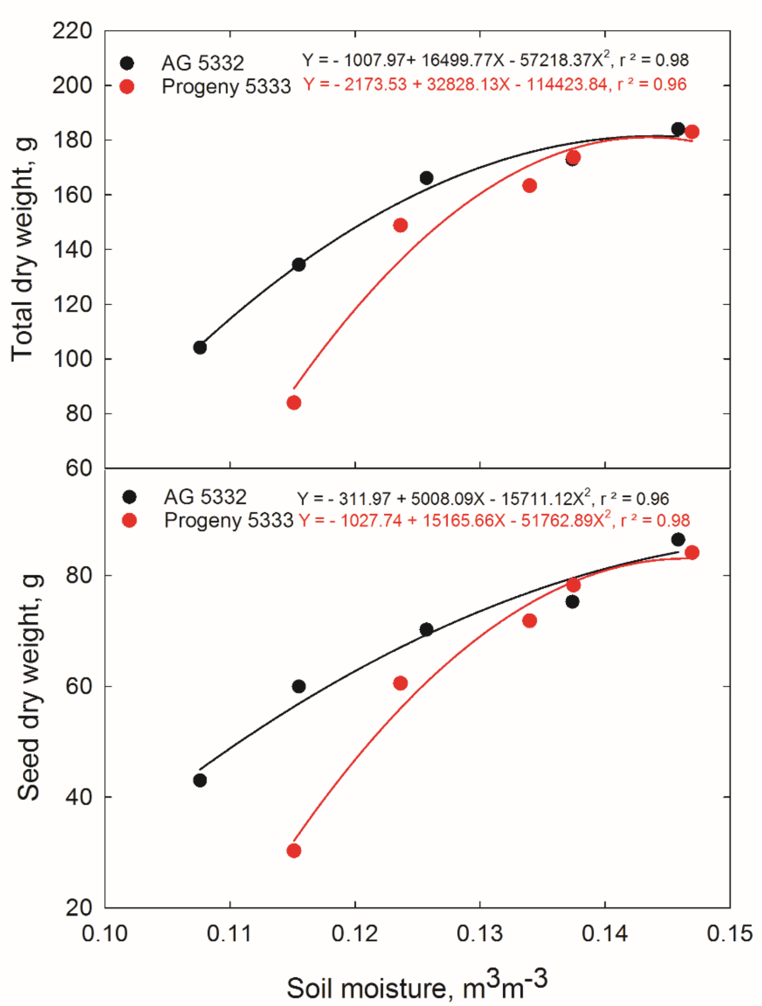


Fig. 22. *Relationship between soil moisture content and total dry weight and seed weight for two soybean cultivars.*

EXPECTED RESULTS

It is expected that this study will be helpful to identify the variation in response to moisture deficit between soybean cultivars that are commonly grown in the Midsouth. The identified tolerance between the soybean cultivars and the responses under each irrigation level will be helpful for the soybean producer to optimize yield through proper management practices and scheduling irrigation during the growing season. Also, the quantified responses to soil moisture stress will be vital to researchers to develop a model based decision support system that is capable of predicting yields based on soil moisture variables.

Student recruitment: 2 Ph.D. students are on-board working on various aspects of the project. One of the students is funded by another agency.

PROJECT DELIVERABLES

Professional meetings:

- **Oral Presentation:** Soil moisture stress effects on yield component distribution in soybean. Chathurika Wijewardana and K. Raja Reddy, SAAS, San Antonio, TX, Feb. 7-9, 2016.
- **Poster Presentation:** Soil Moisture Stress Effects on Root Architecture and Vegetative Growth of Soybean. Chathurika Wijewardana and K. Raja Reddy, SAAS, San Antonio, TX, Feb. 7-9, 2016.
- **Poster Presentation:** Soil moisture stress effects on soybean yield. Chathurika Wijewardana and K. Raja Reddy, MAS meeting, University of Southern Mississippi, Feb. 18, 2016.
- **Oral Presentation:** Reproductive Growth, Development, and Yield Responses of Two Contrasting Soybean Cultivars to Temperature. Firas A. Alsajri and K. Raja Reddy, SAAS, San Antonio, TX, Feb. 7-9, 2016.
- **Poster Presentation:** Reproductive Growth, Development, and Yield Responses of Soybean to Temperature. Firas A. Alsajri and K. Raja Reddy, MAS meeting, University of Southern Mississippi, Feb. 18, 2016. **Second Place Award.**

Table 1. Soil moisture stress induced changes in vegetative, gas exchange, and root traits of soybean. The measurements were taken 29 days after planting. The cultivars used in the study were Asgrow 5333 (AG) and Progeny 5332 (PR).

Cultivar	AG	PG	AG	PG	AG	PG	AG	PG	AG	PG
Parameter / Water stress treatment, % ET	**100**		**80**		**60**		**40**		**20**	
Vegetative traits										
Plant height, cm	15.76 bcd	21.52 a	15.61 cd	18.51 b	13.27 de	16.57 bc	11.72 e	13.67 de	12.2 e	15.42 cd
Leaf no., $plant^{-1}$	7.11 bc	7.67 a	7.00 bcd	7.33 ab	6.88 bcd	7 bcd	6.55 de	6.66 cd	6.11 e	6.77 cd
Leaf area, cm^2	1216.62 b	1417.48 a	791.04 cd	926.88 c	657.86 d	767.66 d	462.99 e	411.76 e	372.28 e	415.37 e
Leaf dry weight, g	3.57 a	3.97 a	2.58 bc	2.78 b	2.30 c	2.47 bc	1.69 d	1.147 d	1.48 d	1.49 d
Stem dry weight, g	2.26 a	2.42 a	1.53 bc	1.66 b	1.33 c	1.39 bc	0.93 d	0.77 d	0.84 d	0.86 d
Shoot dry weight, g	5.83 a	6.4 a	4.11 bc	4.44 b	3.64 c	3.87 bc	2.63 d	2.25 d	2.33 d	2.35 d
Root dry weight, g	1.61 a	1.61 a	1.53 ab	1.43 abc	1.52 ab	1.35 bcd	1.19 cd	0.88 e	1.13 de	0.93 e
Total dry weight, g	7.45 a	8.01 a	5.65 b	5.88 b	5.16 b	5.22 b	3.83 c	3.14 c	3.46 c	3.29 c
Root/shoot	0.28 gh	0.25 h	0.37 de	0.32 fg	0.42 bc	0.34 ef	0.45 ab	0.39 cd	0.49 a	0.39 cd
Gas Exchange Traits										
Pn, µmol CO_2 m^{-2} s^{-1}	25.77 a	25.00 a	24.30 a	23.47 ab	21.70 b	20.90 bc	18.87 cd	21.30 bc	17.00 de	15.77 e
SC, mol H_2O m^{-2} s^{-1}	1.10 a	1.06 a	0.95 b	0.84 c	0.81 cd	0.75 d	0.80 cd	0.66 e	0.75 d	0.58 f
Ci/Ca	0.85 c	0.85 c	0.88 a	0.88 a	0.87 ab	0.88 ab	0.87 b	0.88 ab	0.88 ab	0.88 ab
ETR, µmol m^{-2} s^{-1}	257.39 ab	247.88 b	239.25 ab	222.85 ab	233.87 ab	220.47 ab	218.22 ab	208.49 b	210.05 b	207.99 b
Trans, mmol H_2O m^{-2} s^{-1}	10.05 a	9.37 ab	9.66 a	8.29 c	8.74 bc	8.43 c	8.27 c	8.17 c	7.25 d	7.02 d
Fv'/Fm'	0.563 a	0.56 a	0.54 ab	0.54 ab	0.54 a	0.54 abc	0.53 abcd	0.51 bcd	0.49 d	0.49 cd
Root traits										
Length, cm	12727.82 a	11076.18 ab	10052.4 bcd	9058.99 bc	9239.78 bcd	9055.21 bcd	8197.65 d	7309.26 cd	7222.64 d	7163.33 d
Area, cm^2	1612.29 ab	1420 a	1301.59 bc	1225.58 bc	1254.99 bc	1183.26 bcd	1069.75 d	944.57 cd	957.88 d	953.42 d
Diameter, mm	0.53 ab	0.49 a	0.46 bc	0.43 abc	0.43 bc	0.41 c	0.41 c	0.41 c	0.42 bc	0.42 bc
volume, mm^3	16.31 ab	14.65 a	13.49 bc	13.26 abc	13.6 abc	12.35 bcd	11.14 d	9.74 cd	10.04 d	10.20 d
Tips no., $plant^{-1}$	20432.88 ab	17453.44 a	14382.11 bc	13343.67 bc	15195.33 bc	13771.56 bc	15064.55 bc	14709.55 bc	14496.11 bc	12416.22 c
Forks no., $plant^{-1}$	119933 ab	97707.33 a	92206.44 bcde	87388.11 bcd	94677.67 bc	88695.67 bcde	80455.67 e	67901.78 bcde	73202.22 de	69576 cde
Crossings no., $plant^{-1}$	13388.44 ab	10804.22 a	10034.44 bcd	8690.00 bc	9205.67 bc	9059.67 bcd	7572.56 de	6465 cde	6563.33 e	6067.56 de

Data represent the mean values of nine replicates. Within a row, mean values followed by different letters are significantly different at P = 0.05. The measured gas exchange traits are, Pn; net photosynthesis, SC; stomatal conductance, Ci/Ca, ratio of internal to external CO_2 concentration in the leaf, ETR; electron transport rate, Trans; transpiration rate, Fv'/Fm'; quantum efficiency or fluorescence.

Table 2. Water stress induced changes in vegetative and physiological traits of two soybean cultivars at R3 stage. The cultivars used in the study were Asgrow 5333 (AG) and Progeny 5332 (PR).

	Cultivar									
	AG	**PG**	**AG**	**PG**	**AG**	**PG**	**AG**	**PG**	**AG**	**PG**
	Water stress treatment, % ET									
Parameter	**100**		**80**		**60**		**40**		**20**	
Vegetative traits										
Plant height, cm	122.11 a	107.66 b	117.11 a	99.55 b	111.88 a	98.66 b	98.55 a	95.22 a	75.66 a	79.55 a
Leaf no., plant^{-1}	21.11 a	16.11 d	20.88 a	16.55 d	19.22 b	16.5 d	18.55 bc	16.11 d	17.66 c	15.11 d
Leaf area, cm^2	7801.5 dc	11818.59 a	7357.7 dc	10380.54 ab	7214.07 de	9039.85 bc	5829.1 e	7690.54 dc	4103.97 f	5736.55 fe
Pod no., plant^{-1}	79.78 a	13 b	75.33 a	17.33 b	74.33 a	21.67 b	73.88 a	17.22 b	69.22 a	27.66 b
Leaf dry weight, g	26.8 de	40.94 b	27.9 cde	36.65 ab	25.71 e	34.96 abc	24.47 ef	33.07 bcd	17.69 f	25.73 e
Stem dry weight, g	49.96 bcd	66.5 a	44.95 cde	55.69 b	41.07 def	51.08 bc	37.85 ef	45 cde	25.1 g	32.32 fg
Root dry weight, g	9.89 c	16.27 a	9.91 c	14.62 ab	9.33 c	14.82 ab	12.4 bc	14.1 ab	9.52 bc	10.33 c
Pod dry weight, g	3.97 b	0.24 c	3.16 b	0.15 c	3.68 b	0.46 c	7.38 a	0.5 c	6.42 a	0.69 c
Total dry weight, g	90.64 bcd	123.95 a	85.92 cde	107.11 ab	79.79 de	101.32 bc	82.11 cde	92.68 bcd	58.73 f	69.08 ef
Physiological traits										
Chlorophyll a, µg cm^{-2}	27.25 ed	25.12 abc	22.21 cde	21.95 e	21.49 cde	21.08 de	21.41 ab	20.62 bcd	20.92 a	20.46 bcd
Chlorophyll b, µg cm^{-2}	12.66 c	12.8 bc	12.28 bc	11.62 c	11.71 c	10.94 c	11.44 ab	10.7 bc	10.79 a	10.05 bc
Carotenoids, µg cm^{-2}	5.72 bc	5.53 a	5.79 bc	5.93 bc	6.27 abc	6.31 c	6.33 ab	6.75 bc	7.08 a	6.88 bc
Wax, mg	0.44 e	0.46 de	0.51 cd	0.53 cd	0.52 cd	0.56 bc	0.62 ab	0.61 ab	0.62 ab	0.66 a
Relative injury, %	48.28 abc	44.38a	50.24 a	51.66a	54.72 ab	54.18 bc	55.80 abc	56.67 c	56.78 abc	57.60 bc

Data represent the mean values of nine replicates. Within a row, mean values followed by different letters are statistically different at P = 0.05.