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2017

2016/2017 National Peanut Board/SPRI Proposal
FINAL Report
UF Project: P0042502- PID182-SID:FL114-BID:1543

I. Identification

Title: Utilizing Stress Inoculated Resistance to Manage Drought and TSWV in Peanut
Funding year: January 1, 2017 – June 30, 2018
Investigators: Diane Rowland, Erin Doughtie (dlrowland@ufl.edu; 229-869-2952), and Barry Tillman
Total funds requested: $5,674.98
Locations: Florida
New or Continuing Project: New, year 1

II. Layman's Summary

Peanut production in the southeastern United States is likely to be more frequently affected by periods of drought due to changes in global climate patterns as seen in recent years. These same changing climate patterns are expected to compound water scarcity stress through increasing disease pressure, particularly for viral diseases. For this reason, it is critical to determine practical applications for growers to manage and minimize plant stress. Ultimately, by increasing overall crop stress tolerance, optimization of productivity under a new stressful "norm" is possible. Management solutions for plant stress are varied, but a common approach may be through the utility of stress inoculated resistance (SIR). In SIR, common crop stress defenses and processes may be upregulated through the use of chemical elicitors (Roberts and Taylor, 2016). These elicitor compounds lead to acclimation of the crop and provide improved defenses, particularly to stresses that occur during reproductive developmental periods. By utilizing chemical elicitors, growers may be able to condition the crop to better withstand stress (biotic or abiotic) later in the season when yield losses to stress would be greatest.

III. Project Purpose

Some products that may be involved in eliciting SIR include antitranspirants for drought stress and the use of Thimet® (AMVAC Corporation) for *Tomato spotted wilt virus* in peanut. Both these chemicals have been shown to elicit either stomatal limitations (antitranspirants) or other physiological responses related to disease tolerance in TSWV (Rowland et al., 2005). However, research is needed to elucidate the concept of SIR including: the particular mechanisms of SIR; other chemical elicitors that may be useful for peanut stress tolerance; whether these elicitors can be utilized for cross-stress acclimation (leading to acclimation across a range of stress types); or the variability in SIR among peanut cultivars. While some research has taken place on the application of antitranspirants to peanut (Wilson et al., 2004), the major focus has been on their use in relation to integrated pest management strategies. Our research will focus on the application of antitranspirants as a means to control water loss from transpiration leading to priming of peanut for increased drought tolerance. Thimet is also a purported chemical elicitor known to provide protection from TSWV not solely from thrips control, but likely from some secondary physiological response to phorate that is related to stress priming. Therefore, our project aims to test multiple peanut genotypes cultivated across the southeast by exposing them to 4-5 chemical elicitors known to be active in upregulating stress responses. The aim is to determine which pairings of genotype and chemical stress elicitor have the highest net positive effect on overall plant health, physiological performance, and yield under TSWV infection and drought conditions.

IV. Hypotheses and Objectives

The primary hypothesis of this study is that chemical elicitors have the potential to enhance peanut tolerance to abiotic and biotic stress through mechanisms of SIR. Specifically, we hypothesize chemical elicitors: 1) will enhance priming for water deficit stress, thus improving tolerance to drought during mid-season; 2) may lead to improved TSWV resistance through exposure to mild levels of physiological stress; and 3) may lead to cross acclimation such that resistance to drought and disease may occur.

V. Experimental Plan and Methods

Field experiments will be established at the Plant Science Research and Education Unit (PSREU) in Citra, FL for a combined test of drought and TSWV elicitors, and a second field experiment will be established at the North Florida Research and Education Center (NFREC) in Marianna, FL to test TSWV elicitors. The PSREU site is appropriate for a combined test of elicitors because of its variable rate irrigation system and moderate level of historical TSWV infection levels; while NFREC has the highest TSWV pressure. At both sites, replicated tests utilizing randomized complete block designs will be implemented. At PSREU, main plots will consist of chemical elicitor treatments and sub plots will consist of peanut cultivars; for the PSREU site, main plots will be water deficit stress level, sub plots will be chemical elicitors, and sub-sub plots will be genotype. We will use plot based spray programs applied to six cultivars including TufRun511, COC041, EP113 - all genotypes known to have variability in stress priming potential. Measurements will include in-season physiological assessment, particularly OJIP chlorophyll fluorescence for quantifying stress impacts on photosystem II; TSWV disease progression through ELISA testing of root crowns; and canopy development; while end season measurements will include yield and grade.

VI. Measurable Outcomes and Potential Impact

At the end of these experiments, we expect to have data quantifying the SIR potential of various chemical elicitors in peanut and determine their effectiveness in addressing cross-stress priming. In the context of drought stress, this information has the potential to provide producers with chemical elicitors that improve water scarcity tolerance. For TSWV, the disease continues to be a significant threat to the peanut industry with the potential to reduce production by 50% or more in a single season. If peanut genotypes can be found that can be chemically stressed to inoculate against the virus providing some resistance, a large proportion of loss could be mitigated. This would provide growers chemical elicitors that may provide some level of protection to the disease not related to thrips control. Overall, understanding the process of stress defense mechanisms imparting SIR will provide stress management strategies to growers for combatting climate uncertainty.

VII. Potential Pitfalls

There is potential that the chemical elicitors tested may cause negative yield impacts; however, this is part of the overall quantifiable objectives being addressed and will be evaluated at harvest. Other challenges may be related to concentrations and application timings for the purported chemical elicitors to achieve stress priming. For our purposes, we will begin this research utilizing the recommended product concentration and application timings from manufacturers and explore questions of varying product quantity after identifying the most promising chemistries.

VIII. Budget (see attached Excel sheet)

References:

Roberts MR, and Taylor JE. 2016. Exploiting plant induced resistance as a route to sustainable crop protection. *In* DB Collinge (ed.), Plant Pathogen Resistance Biotechnology, John Wiley & Sons, Inc.

Rowland D, Dorner J, Sorensen R, Beasley JP Jr., and J Todd. 2005. Tomato spotted wilt virus in peanut tissue types and physiological effects related to disease incidence and severity. Plant Pathology 54: 431-440.

Wilson JP, CC Holbrook, B Mandal, DL Rowland, ML Wells, and DM Wilson. 2004. Efficacy of Foliar Applications of Particle Films and Genotype for Managing Thrips, Diseases, and Aflatoxin in Peanut. Plant Health Progress doi:10.1094/PHP-2004-0419-01-RS.

FINAL REPORT

I. Introduction & Hypotheses

This trial was set up to examine whether a series of chemical priming agents could be used to improve the drought tolerance of peanut in a single growing season. We selected three products to test against a control to determine the impact on physiological development, yield and photosynthetic efficiency. The main hypotheses were a) that all treatments would have some impact on the physiological development of the crop, b) that the AMF treatment would see the greatest reduction in yield from drought as compared to other treatments, and c) that the AMF treatment would exhibit the greater root development under rainfed and irrigated conditions as compared to the control treatment. A series of measurements, described in the following section, were taken along with yield data that will be collection upon harvest in late October and early November of 2017.

II. Methods

The trial was planted on May 23, 2017 at the Plant Science Research and Education Center, a research facility of the University of Florida, located in Citra, FL. The trial was a complete randomized block design in split plot arrangement consisting of a total of 64 plots that were approximately 9.144 meters in length with four rows in each plot. There were two irrigation regimes including a rainfed and an irrigated treatment. The threshold to apply irrigation was defined at 40 kPa of soil water tension using irrometers installed at a depth of 15.24 cm. When the trigger point was reached under this protocol, 19 mm of water would be applied using a linear irrigation system. Four plant growth promoting treatments, including the control, were applied which included ProGibb, a gibberellin-based, Maxcel, a cytokinin-based product and MycoApply EndoPrime an endomycorrhizal (AMF) product. One peanut variety, TUFRunner™ '511' variety (U.S. PVP No. 201400249), was selected which is a 140 maturity high-oleic runner type. Previous drought studies in peanut have found this variety to be able to be droughted, yet not overly sensitive to drought making it an ideal candidate for this study. Treatments were applied as shown in Table 1. Four replications were included in the trial design. The soil type is a Hague sand, essentially beach sand, with adequate drainage making it ideal for drought studies.

Product	Application Rate	No. of Apps	Method
Maxcel	25ppm @ 100L/ha	2	Foliar Spray
ProGibb	80ppm @ 100L/ha	2	Foliar spray
MycoApply Endoprime	1tbsp/gal/50ft^2	1	Soil Drench

Table 1. Application rates of plant growth promoting treatments

The ProGibb and Maxcel treatments were applied at the appearance of two or more leaves in 65-70% of the crop, 10 and 20 days following the initial application. This protocol for both products was inherited from Dr. Paxton Payton at USDA-ARS in Lubbock, TX. The MycoApply EndoPrime was applied via a soil drench at two days after planting to ensure establishment in line with the development of the crop.

A series of physiological measurements were taken to quantify the rate of physiological development of peanut in response to the treatments and irrigation. These measurements included leaf area index (LAI), the ratio of light intercepted by the crop canopy, and minirhizotron imaging to examine root development between the AMF and control treatments. Additional measurements were taken including OJIP chlorophyll fluorescence which is used to detect the impact of stress in photosystem II and gas exchange to quantify photosynthetic efficiency. LAI was taken using LI-COR LAI-2200 leaf canopy analyzers on three dates over the season until full canopy closure was reached in 75-80% of the crop. Gas exchange was taken using LI-COR IRGA-6400XT on two dates while OJIP was taken on dark-adapted leaves using Opti-Sciences OS30+ chlorophyll fluorometers on four dates until the crop canopy has closed. Rhizotron imaging was taken on six dates determined by the application dates of the treatments and critical crop growth stages. Tubes were installed at approximately two weeks after planting at 45 degree angle centered under the crop (see Figure 2). Images were analyzed using WinRhizo software to examine total root length and surface area in each plot. Thirty-two total tubes were installed with one tube in each of the sixteen AMF plots and one tube in each of the control plots.

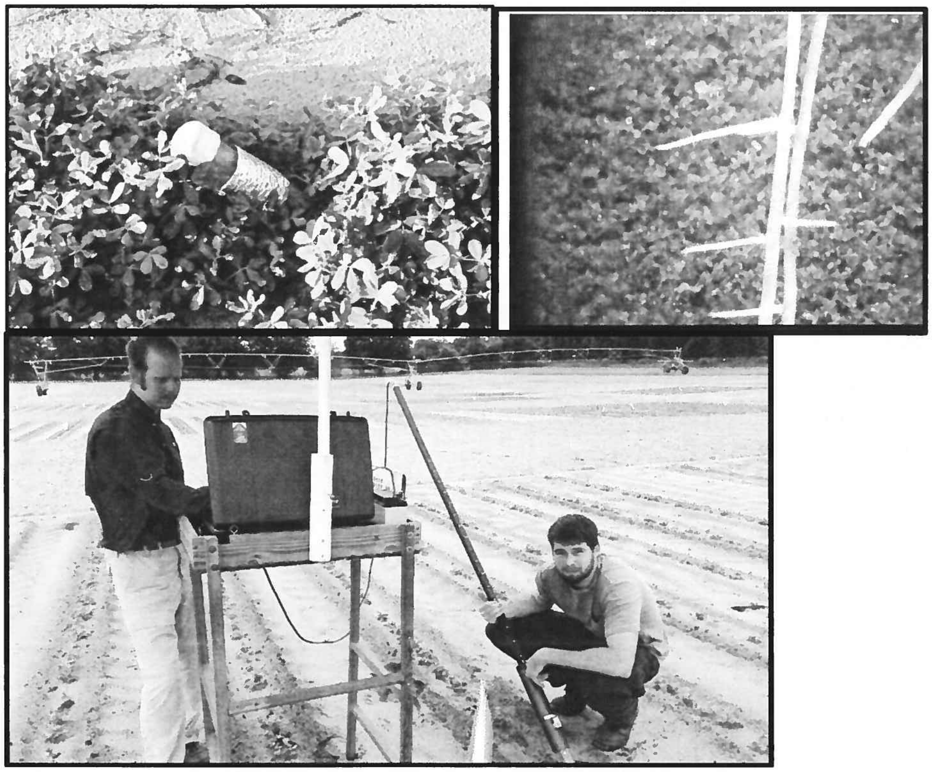

Figure 1. From top left: installed tube with PVC cap, a root image from Plot 111 at 37 DAP, student workers during an imaging session.

III. Results

During the 2017 growing season Citra, FL experienced the 4th wettest year on record with 140.75cm of rainfall from May 23rd until October 10th, 2017 compared to an annual average over the same time period of between 67-70cm according to NOAA. As a result of high rainfall, no irrigation was applied in 2017. Rainfall information is provided in Figure 2.

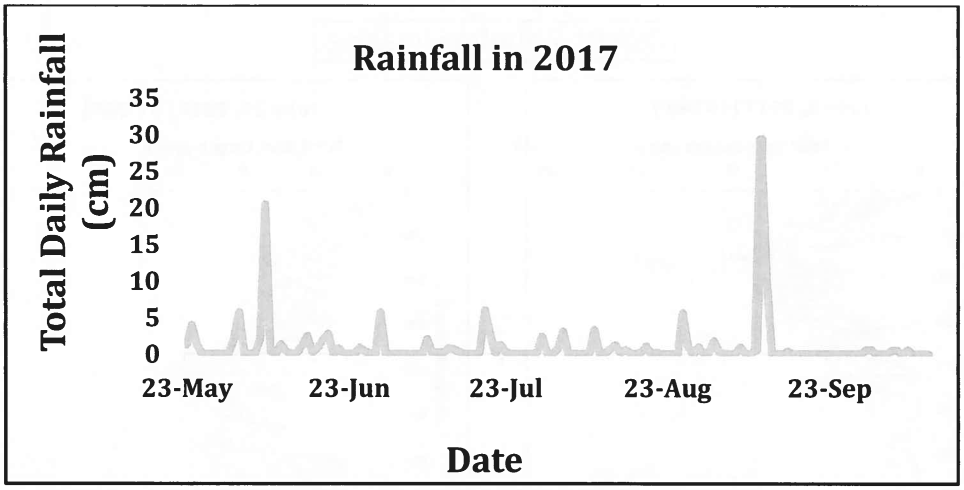

Figure 2. Daily Total Rainfall May 23 to October 10, 2017

Measurements of LAI was taken at 28, 59 and 78 days after planting and showed no significant differences. This is an unanticipated result because ProGibb typically elongates stems in other crop species and might be expected to exhibit greater LAI than other treatments. The lack of significant difference is likely due to the higher rainfall over the season which could have either washed off foliar treatments prior to them taking effect or could have confounded treatment effects by leeching nutrients from the soil. Chlorosis of the leaves was observed at approximately 40 DAP and attributed to nutrient loss from the soil and fertilization was applied more frequently as a result and mitigated some of the chlorosis within one week after application. Measurements of OJIP were taken at 20, 30, 60 and 90 days after planting between 3am and 5:30am to ensure full dark adaptation on two leaves per plot across all treatments. Initially we looked at parameters that describe performance, absorbance and trapping as these usually show some indication of treatment differences in the photosynthetic efficiency of plants, however we did not find any significant results in these cases. We did however find a treatment effect for the parameter RC/CSm which refers to the number of photosystem II reaction centers that are active per cross section. A reaction center is defined as, "…a group of electron transfer proteins that receive energy from the antenna complex and convert it to chemical energy using oxidation reductions," (Taiz & Zeiger, 2010). This would indicate that other parameters should also be investigated to determine if there are other treatment differences, and what those differences are. At present we have collected the data on a variety of OJIP parameters which is currently be analyzed.

Root imaging data using minirhizotrons was collected at 16, 23, 37, 51 65 and 86 days after planting and was manually traced using WinRhizo software. Thus far we have only been able to analyze two dates to look for significant differences between treatments. Other data for the other four dates is forthcoming following harvest on

October 30th. Root data for the first and third imaging sessions is shown below in Figure 3. Given this limited data is appears that in the first imaging date that AMF plots show greater total root length than the control at shallower soil depths, however this difference appears to fade over time by 37 days after planting. Further analysis of the remaining imaging session is needed to determine if there are any significant differences in total root length and root surface area per treatment.

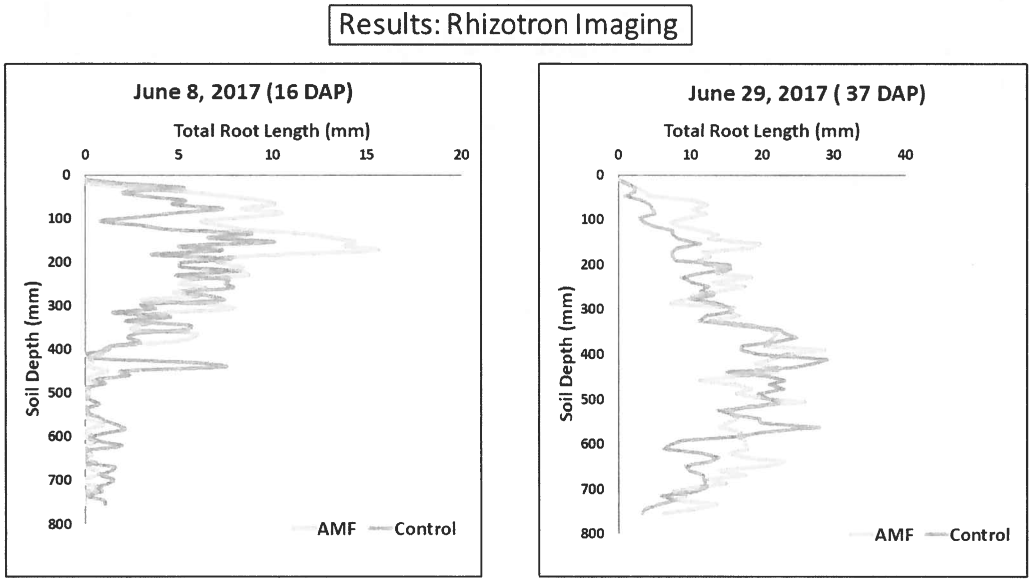

Figure 3. Root data at 16 and 37 days after planting comparing AMF and control plots by total root length by soil depth.

We also took measurements of gas exchange at 61 and 86 DAP that requires further analysis to draw any substantive conclusions. In addition, sixteen soils cores were taken between 90-95 days after planting in each of the AMF treated plots. The cores are approximately 36 centimeters long and 8.26 centimeters in circumference and will be divided into four cores each of approximately 9 centimeters in length. We will then harvest ten root segments of approximately 5.08 cm in length each and stain them using the gridline intersection method. We will then examine stained roots under a microscope to quantify the level of colonization by core depth to see how effective the treatment was and ensure any results are not due to field conditions.

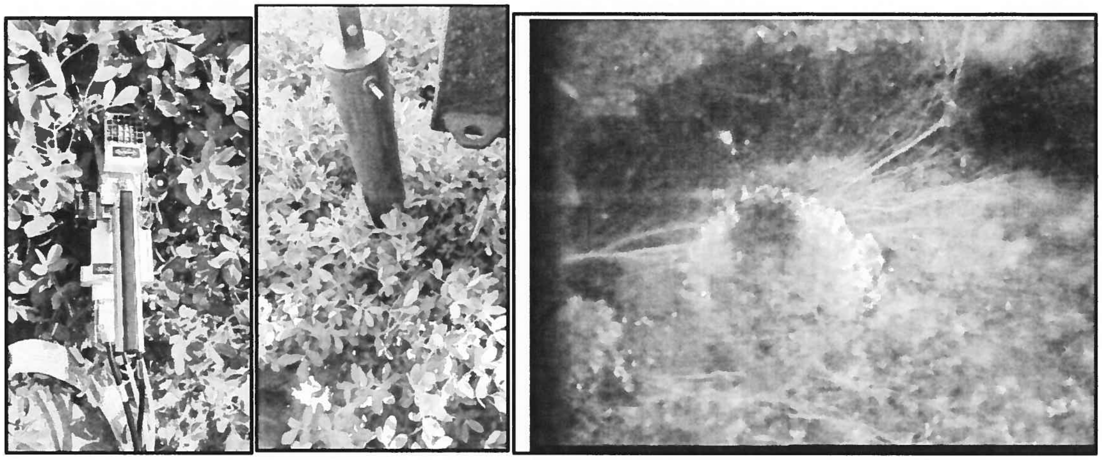

Figure 4. From left to right: IRGA measurements of gas exchange, soil core collection in AMF plot, and image of mycorrhizae from root imaging session at 51 DAP.