Clemson University Final Project Report (December 18, 2023)

<u>Project title</u>: From nursery to field: Arbuscular mycorrhizae fungi associated management of Fusarium wilt in watermelon

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Introduction

An on-farm research trial was started (April 10, 2023) at a farmer field in Barnwell County, SC that had history of severe Fusarium wilt in watermelon in 2022. The following treatments were implemented in the experiment.

Treatments

1. 50 Lbs. of recommended P_2O_5 + Nursery without AMF
2. 50 Lbs. of recommended P_2O_5 + Nursery with AMF
3. No P + Nursery without AMF
4. No P + Nursery with AMF
5. 50 Lbs. of recommended P_2O_5 + Carolina Strongback grafted watermelon nursery

The arbuscular mycorrhizae fungi (AMF) inoculum (Sp. *Rhizophagus irregularis*) was purchased from INVAM (The University of Kansas, Lawrence KS). The watermelon cultivar (*LaJoya*) seed was purchased early February 2023. Nutrient free potting mix was purchased form Berger and steam autoclaved to kill any residual microbes and pathogens. The watermelon nursery was planted on March 1, 2023, as per the recommended guidelines for planting watermelon nursery by Clemson University. One ml of AMF inoculum was applied per cell (per plant) in AMF treated nursery. A special pollinizer variety (SP7) of watermelon was also planted. The nursey was managed in greenhouse at the Edisto Research and Education Center (EREC), Clemson University. Starter nitrogen (N) and potassium (K) were applied as per soil testing recommendations through 15:0:15 and phosphorus (P) fertilizers (100 % P based on soil testing recommendations by Agricultural Services Laboratory, Clemson University) through triple super phosphate as per treatments. Pre-transplanting fertilizers were thoroughly incorporated into soil, on raised beds. The watermelon nursery was transplanted on the raised beds with plastic mulch at row spacing of 6 ft and 4 ft plant spacing within rows. The experiment had 5 treatments, each replicated 4 times on a plot size of 36×20 ft^2 (6 rows with 5 plants in each row). A pollinizer watermelon variety (SP7) seedling was transplanted after every 3 seedless watermelon plants in the row. Drip irrigation and fertigation system were installed at the time of laying plastic, and soluble fertilizer program was initiated as pre recommendations by Southeastern US vegetable handbook.

We also conducted a second small field experiment at EREC, Clemson university with 4 treatments each replicated 5 times. The soils at EREC are not infested with fusarium wilt so the purpose of the experiment at EREC was to evaluate the impact of AMF and P fertilizer on watermelon P use efficiency, plant growth, root AMF colonization and root growth. All other crop management practices were similar to as specified above.

Treatments

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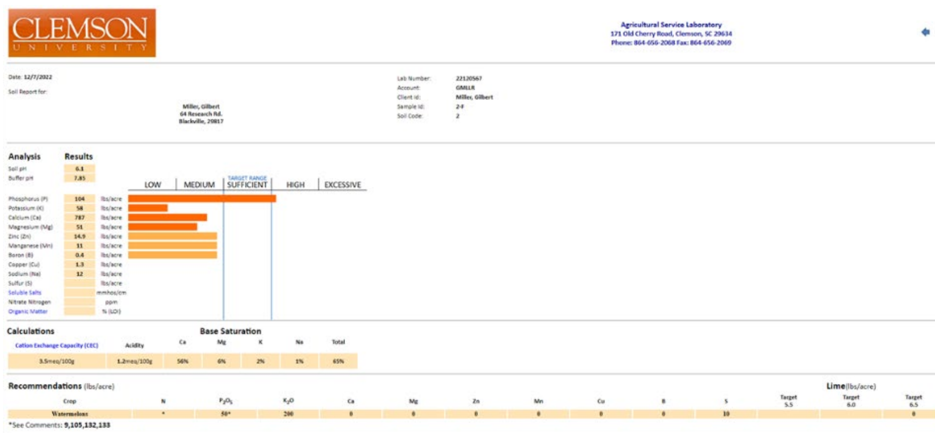
2. 50 Lbs. of recommended P_2O_5 + Nursery with AMF
3. No P + Nursery without AMF
4. No P + Nursery with AMF

Results

The soil samples were submitted for nutrient analysis to Agricultural Services Laboratory, Clemson University. The soil (from on-farm experiment) had sufficient to high in P, low in K, medium in calcium (Ca) and magnesium (Mg) (Figure 1). Agricultural services laboratory recommended to apply 50 pounds of P_2O_5 and 200 pounds K_2O.


CLEMSON UNIVERSITY

Agricultural Service Laboratory
171 Old Cherry Road, Clemson, SC 29634
Phone: 864-656-2068 Fax: 864-656-2069

Date: 12/7/2022

Soil Report for:

Miller, Gilbert
64 Research Rd.
Blackville, 29817

Lab Number: 22120567
Account: GMILLR
Client id: Miller, Gilbert
Sample id: 2-F
Soil Code: 2

Analysis	Results	
Soil pH	6.1	
Buffer pH	7.85	
Phosphorus (P)	104	lbs/acre
Potassium (K)	58	lbs/acre
Calcium (Ca)	787	lbs/acre
Magnesium (Mg)	51	lbs/acre
Zinc (Zn)	14.9	lbs/acre
Manganese (Mn)	11	lbs/acre
Boron (B)	0.4	lbs/acre
Copper (Cu)	1.3	lbs/acre
Sodium (Na)	12	lbs/acre
Sulfur (S)		lbs/acre
Soluble Salts		mmhos/cm
Nitrate Nitrogen		ppm
Organic Matter		% (LOI)

Calculations

Cation Exchange Capacity (CEC)	Acidity	Base Saturation Ca	Mg	K	Na	Total
3.5meq/100g	1.2meq/100g	56%	6%	2%	1%	65%

Recommendations (lbs/acre)

Crop	N	P_2O_5	K_2O	Ca	Mg	Zn	Mn	Cu	B	S	Lime (lbs/acre) Target 5.5	Target 6.0	Target 6.5
Watermelons	*	50*	200	0	0	0	0	0	0	10			0

*See Comments: 9,105,132,133

Figure 1: *The soil nutrient analysis from on-farm research fields. Soil samples were collected and analyzed before the start of the experiment.*

The watermelon nursery was managed in the greenhouse for 40 days and 40 days old nursery was transplanted into field. Nursery before transplanting was analyzed for the root mycorrhizae colonization and tissue P content. The AMF inoculated nursery had 49.2% root mycorrhizae colonization as compared to 0% in non-AMF inoculated nursery (Figure 2a). Which means 49.2% of the root surface was colonized by the mycorrhizae fungi in AMF inoculated nursery. The AMF inoculated nursery had slightly higher tissue P concentration (0.317%) as compared to non-AMF (0.301%) nursery (Figure 2b).

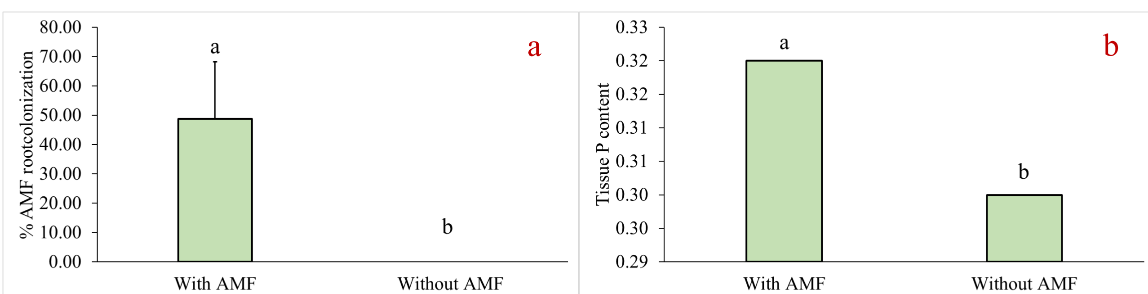


Figure 2*: Percent root mycorrhizae colonization (a) and percent tissue P content (b) of 5-week-old watermelon nursery as affected by nursery AMF inoculation in potting mix.*

On-farm research experiment:

Disease severity index: Disease severity index (DSI) was calculated as per the method described in the proposal write up. First symptoms of fusarium wilt in watermelon were seen 28 days after transplanting the nursery after which DSI was calculated on weekly intervals. At 28 days, we observed around 20% DSI in all treatments except for strongback grafted watermelon treatment. The DSI increased to 40% at 35 days, to 59% at 42 days and reached above 90% at 49 and 54 days after transplanting (Figure 3). The DSI in strong back grafted watermelon was less than 10% throughout the crop duration. In all treatments except for strongback grafting, we observed complete mortality of the crop. The AMF and P treatments had no effect on the fusarium wilt management in watermelon under on-farm field conditions.

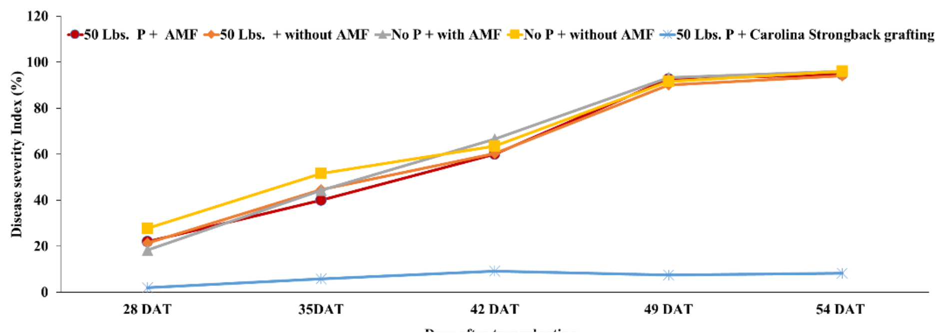


Figure 3*: The effect of AMF inoculation, P fertilizer application and strongback grafting on the fusarium wilt disease severity index (%) in watermelon.*

Marketable Fruit yield: Watermelon fruits were harvested on June 29, July 6 and July 13th, 2023. The fruits were immediately moved to field lab and tested for weight, brix, black seeds and hallow heart to match the USDA marketable watermelon grades. A watermelon with brix reading less than 9, weight less than 7 lbs and/or with hallow heart was eliminated from the marketable yield. Due to high disease severity index in all plots except Carolina strong back grafted plots, almost all the plants died before reaching the flowering/fruiting growth stage. A marketable fruit yield of 40,000 lbs was observed in Carolina strong back grafted plots which was significantly higher than all other treatments (Figure 4). We observed no marketable fruit yield in other treatments.

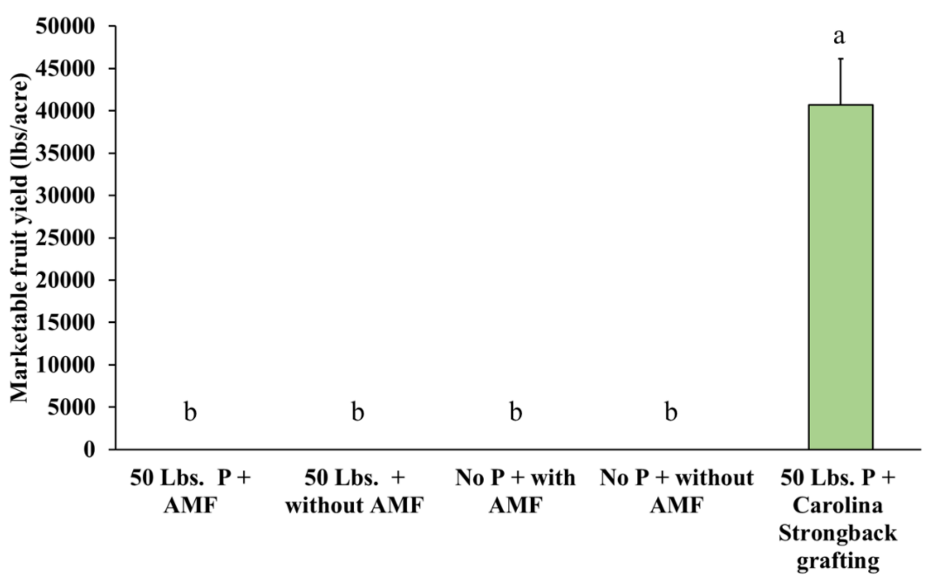


Figure 4*: The effect of AMF inoculation, P fertilizer application and strongback grafting on marketable fruit yield in watermelon.*

Fruit quality: We did not observe any effect of different treatments on Brix and hallow heart in watermelon fruits.

Economic analysis: The economic analysis was conducted by collecting the average market sales price of watermelon in the week of June 30 and July 14th when we harvested the fruits. The average selling price for watermelon of weight between 10-14 lbs was approximately $0.20 per pounds in the week of June 30 and $0.15 in the week of July 14th. Majority of marketable watermelons harvested had weight between 10-14 lbs. The cost of production for watermelon was collected from enterprise budget sheet prepared by Clemson co-operative extension for seedless watermelon (irrigated). The cost of AMF culture was based on the bulk price of the AMF culture to grow nursery and for grafted watermelon nursery was selected as $1.20 per grafted plant. Since we did not had any harvest in all treatments except Carolina strong back grafted nursery plots, the cost of harvesting, pesticides (except herbicide) and other harvesting expenses were excluded from net cost of production except for Carolina Strongback grafted nursery treatment.

With and without AMF + P fertilizer treatments had a net cost of production and less loss of $2790.59 to $2967.86 (Table 1). This was due to no marketable fruit harvest in the treatments. The cost of production of Carolina strong back grafted watermelon was $1592 higher than the regular nursery per acre. A total profit of $1229.68 and benefit: cost ratio of 20.8% was observed in Carolina strong back grafted watermelon nursery (Table 1), which was due to harvest of around 40,000 lbs of marketable fruits per acre.

Table 1: Watermelon enterprise budget as influenced by different treatments.

Treatment	Net cost of production ($)	Gross revenue ($)	Net revenue ($)	Benefit: cost (%)
50 Lbs. of recommended P + Nursery without AMF	2790.59	0	-2790.59	---
50 Lbs. of recommended P + Nursery with AMF	2967.86	0	-2967.86	---
No P + Nursery without AMF	2790.59	0	-2790.59	---
No P + Nursery with AMF	2967.86	0	-2970.86	---
50 Lbs. of recommended P + Carolina Strongback grafted watermelon nursery	5924.90	7154.58	1229.68	20.8

Edisto research and education center (EREC) experiment results:

The above ground biomass and root samples were collected from the watermelon plants after 30 days of transplanting. The above ground biomass was dried, and dry biomass weight was measured. Dry biomass tissues were analyzed for tissue P content. Root samples were analyzed for the percent root AMF colonization. We did not observe any effect of AMF and P fertilizer treatments on the dry biomass accumulation of watermelon (Figure 5a). Tissue P content in No P + No AMF treatment was significantly lower than the 100%P + with and without AMF and No P+ AMF treatment at 30 days after transplanting (Figure 5b). This means that AMF root colonization increased plant's ability to uptake and utilize the soil P reserves. The AMF colonized nursery roots had 25 to 29 percent root colonization in both 100 % P and No P treatments which was significantly higher than without AMF treated nursery (Figure 5c). We did observe 12-15% of root AMF colonization in No AMF treatments which could be due to mycorrhizae inoculum in field soils (Figure 5c).

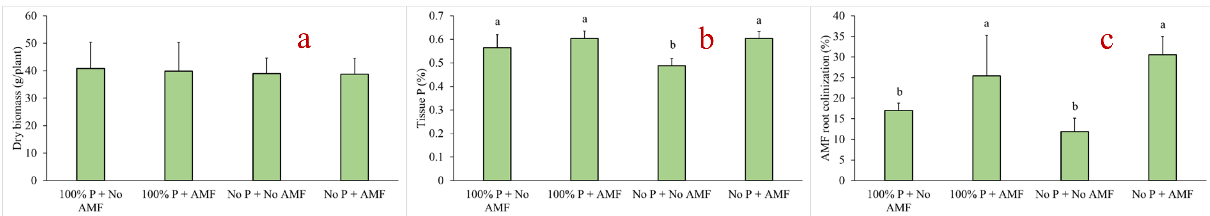


Figure 5: *The effect of different nursery AMF inoculation and P fertilizer treatments on the watermelon aboveground dry biomass (a), tissue P concentration (b) and root AMF colonization (c) at 30 days after transplanting.*

Outcomes: Oral talks was given at the *Edisto REC watermelon field day* (July 13th, 2023) and at annual *American Society of Horticultural Science meeting-2023* (July 29-August 3, 2023), held in Orlando, FL. A *6-month progress report* was submitted to National Watermelon Association and a *magazine article* was published in the Vineline Magazine (National Watermelon Association). Two high school students (Landon Williams and Jarrett Wootten) with interest in Horticulture, were trained in the project.

Jatana BS (2023). Fusarium wilt research at Edisto REC. Watermelon field day, July 13, 2023.

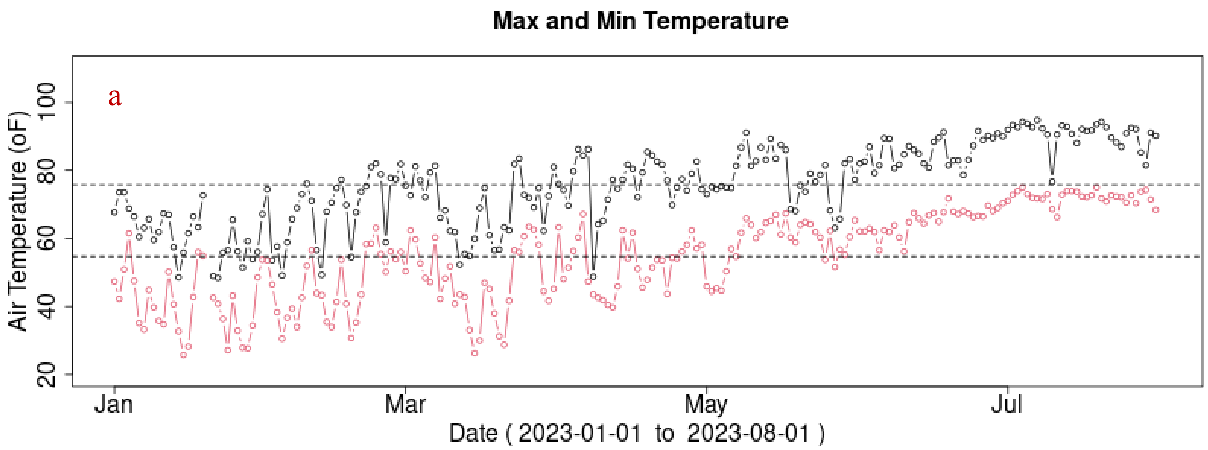
Jatana BS, Miller G and Ray C (2023) Can arbuscular mycorrhizal colonization and phosphorus fertilization alleviate fusarium wilt in watermelon? American Society of Horticultural Science annual meeting, Orlando Florida, July 29- August 3, 2023.

Jatana BS (2023) Fusarium wilt defense in watermelons using arbuscular mycorrhizae fungi and grafted Carolina strongback. Vineline, National watermelon Association

Challenges encountered, unusual developments and conclusions.

Even though reported in literature that AMF help plant survive the root borne disease infection, we did not observe any effect of AMF colonization on fusarium wilt in watermelon. We observed approximately 95% disease severity index and more than 90% plant mortality in watermelon plants (on-farm experiment) with and without AMF inoculated nursery. We also encountered the deer damage problem on some of the experimental plots within first 30 days. After which immediately, we installed an electric fence, which worked well. Since we had more than 90 percent plant mortality, we were not able to analyze the fusarium colony forming units in watermelon roots and rhizosphere soils.

Even though we did not observe any effect of nursery inoculated AMF on fusarium wilt management in watermelon this year, this experiment needs to be conducted for multiple years to make conclusions. This year was exceptionally wet and cold during watermelon growing season as compared to average regional precipitation and temperature (Figure 6), which favors the fusarium wilt infection in watermelon (Keinath et al. 2019, *Plant Disease*). This could be the reason for aggressive nature of fusarium wilt in watermelon this year. In 2024, we will be conducting another experiment with AMF inoculated nursery for fusarium wilt management in watermelon to make conclusions.



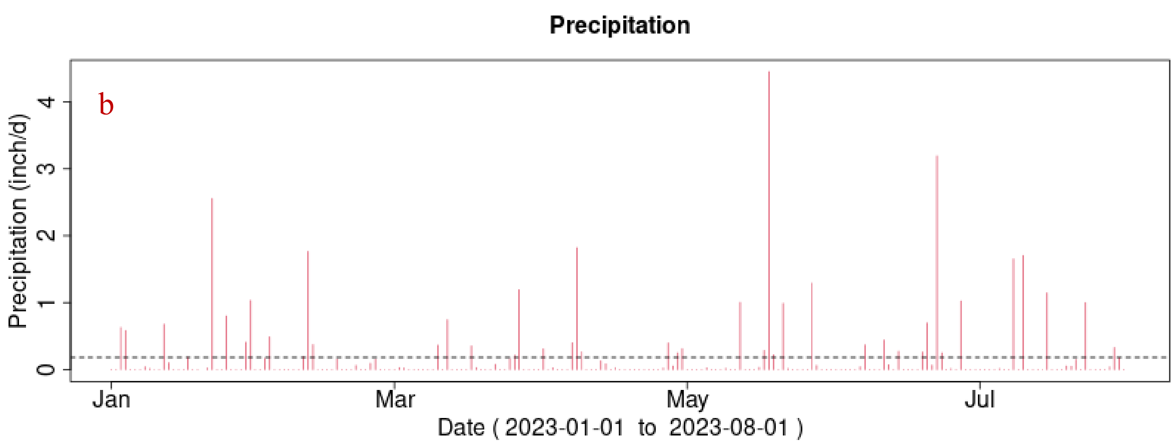


Figure 6: *The daily minimum and maximum temperature (a) and precipitation (b) at Edisto Research and Education Center in 2023 during watermelon growing season.*