Project Title: Characterization of a new bacterial pathogen on watermelon, squash and other cucurbits, understanding the key environmental factors favoring the disease, and development of an IPM strategy

Principal Investigator: Dr. Mathews L. Paret, University of Florida - NFREC, Quincy, FL and Plant Pathology Department, Gainesville, FL

Other Project Investigators and collaborators: Dr. Jeffrey B. Jones, University of Florida – Plant Pathology Department, Gainesville, FL; Dr. Nicholas S. Dufault, University of Florida – Plant Pathology Department, Gainesville, FL; Dr. Joshua H. Freeman, University of Florida – NFREC, Quincy and Horticultural Sciences Department, Gainesville, FL; Bob Hochmuth, University of Florida – NFREC, Live Oak, FL; Dr. Pamela Roberts, University of Florida – SWFREC, Immokalee, FL and Plant Pathology Department, Gainesville, FL; Dr. Carolee T. Bull – USDA, Salinas, CA; Dr. Erica Goss - University of Florida, Plant Pathology Department and Emerging Pathogens Institute, Gainesville, FL; Dr. Eric M. Newberry – University of Florida, NFREC, Quincy, FL and Plant Pathology Department, Gainesville, FL (Graduate student); Dr. Binoy Babu – University of Florida, NFREC, Quincy, FL (Post-doctoral Fellow); Dr. David Langston – University of Georgia, Tifton, GA; Dr. Bhabesh Dutta – University of Georgia, Tifton, GA; Dr. Bert Woudt – Syngenta Seeds, Enkhuizen, Netherlands; Neal Kicklighter – Louis Taylor Farms, Tifton, GA; Dr. Steven C. Bost – University of Tennessee, Nashville, TN

Amount, source of funds and year/s, and equipment or research contributions: Florida Watermelon Association – $6,000 (2013-2014); National Watermelon Association – $9,500 (2015); USDA-Southern IPM Center – $29,966 (2015-2016); Louis Taylor Farms – Design and fabrication of irrigation system for research studies – 2015; Syngenta Seeds Student Internship, Netherlands (Eric Newberry) – 2016

Background: In 2013, a new bacterial disease affected watermelon production in ~6,500 acres in Florida with an estimated yield loss of 5-15% due to severe infection at the early stages of the crop leading to leaf blighting, transplant losses and delayed fruiting of infected plants affecting marketability. Similar issues were also seen in watermelon, squash and other cucurbits in the recent years in California, Georgia, Tennessee and many countries outside U.S.

Key research activities: The objectives of the study were 1) Genetic, morphological, biochemical and pathogenic characterization of novel *Pseudomonas* spp. strains affecting watermelon, squash and other cucurbits 2) Identifying key environmental factors favoring disease occurrence and spread and 3) Evaluating integrated use of copper, mancozeb, Acibenzolar S-Methyl (ASM), a Systemic Acquired Resistance (SAR) Inducer and bio-control agents.

Results of research: Novel strains of *Pseudomonas syringae* affecting watermelon, squash and other cucurbits were identified and characterized. Cool, wet conditions favored disease occurrence and spread of the *Pseudomonas syringae* strains. Copper + Mancozeb was the most effective in reducing disease severity in watermelon compared to the water control under greenhouse and field conditions. ASM and bio-control agents also had some efficacy in reducing disease severity.

References

https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-11-15-1332-RE
https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-11-16-1628-RE
https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-07-17-1002-RE?journalCode=pdis
https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-11-15-1387-PDN?mobileUi=0

Project Title: Characterization of the 2018 disease outbreak on watermelon associated with a diverse group of *Xanthomonas* spp.

Names of PI and others on the project team: Dr. Gary E. Vallad, UF-IFAS, Gulf Coast Research and Education Center, Wimauma, FL; Dr. Peter Abrahamian, UF-IFAS, Gulf Coast Research and Education Center, Wimauma, FL; Dr. Juliana Pereira, UF-IFAS, Gulf Coast Research and Education Center, Wimauma, FL; Dr. Jeffrey B. Jones, UF-IFAS, Plant Pathology Department, Gainesville, FL; Dr. Carrie L. Harmon, UF-IFAS, Plant Pathology Department, Gainesville, FL; Dr. Nicholas S. Dufault, UF-IFAS, Plant Pathology Department, Gainesville, FL; Dr. Mathews L. Paret, UF-IFAS, North Florida Research and Education Center, Quincy, FL; Bob Hochmuth, UF-IFAS, North Florida Research and Education Center, Quincy, FL.

Amount and sources of funds: No external funds were provided for this project.

Background: Unusual foliar symptoms were observed in commercial watermelon fields throughout Florida beginning in April through June of 2018. Yellow, mucoid bacterial colonies characteristic of *Xanthomonas* spp. were consistently isolated from plant samples. Ensuing discussions among project team members led to a coordinated research effort to identify the causal pathogen(s) and develop disease management recommendations for the Florida watermelon industry. Prior reports exist for *X. cucurbitae* and *X. melonis* on watermelon and other cucurbits, but not in Florida.

Brief description of key research activities:

- Collected 16 Xanthomonas strains from symptomatic watermelons throughout Florida, encompassing 8 different counties. Included a historical sample from DPI collected in 2009.
- Performed biochemical, molecular and pathogenic assays, to characterize strains; including strain tolerance to copper and streptomycin.
- Established coordinated field trials in Live Oak and Wimauma to assess impact of disease on watermelon yield and response to several pesticides.

Status/Results of research results to date:

- Biochemical and molecular assays confirmed all strains as *Xanthomonas.* Further strain characterization using a multi-locus sequence analysis (MLSA), based on 6 housekeeping genes, differentiated strains into three distinct species: *X. cucurbitae* (n=1), *X. melonis* (n=4) and *X. arboricola* (n=12).
- Of the 17 strains, 9 and 2 strains tolerated copper sulphate (200 ppm) and streptomycin (200 ppm), respectively, based on their ability to grow on amended media.
- Of the strains tested, only *X. cucurbitae* consistently caused well-defined disease symptoms on plants when spray inoculated or infiltrated into leaf tissues. Others, were only weakly pathogenic, causing some symptoms on various cucurbits and non-cucurbit hosts following leaf infiltrations or when plants were incubated under water-saturated conditions for long periods of time.
- Watermelon field trials at Live Oak and Wimauma were inoculated with a mixture of *Xanthomonas* strains: *X. cucurbitae* (n=1), *X. melonis* (n=2) and *X. arboricola* (n=3). Isolation from symptomatic plants in Live Oak only recovered *X. melonis*; those in Wimauma recovered *X. cucurbitae*. Little disease progress was observed following trial inoculation at either site, so no conclusions can be made for the impact on yield or pesticide efficacy at this time.

Project Title: Development of an improved scouting technique incorporating UAV-Multispectral imaging into conventional practices on Florida cucurbits

Principal Investigator: Dr. Mathews L. Paret, University of Florida - NFREC, Quincy, FL and Plant Pathology Department, Gainesville, FL

Other Project Investigators and collaborators: Dr. Joshua H. Freeman, University of Florida – NFREC, Quincy and Horticultural Sciences Department, Gainesville, FL; Dr. Charles Mellinger – Glades Crop Care, Jupiter, FL; Darren Raj; Agribugs, Tallahassee, FL; Dr. Jnaneshwar Das, Arizona State University, Tempe, AZ; Bob Hochmuth, University of Florida – NFREC, Live Oak, FL; Dr. Jim Marois, Glades Crop Care, Dr. Melanie Kalischuk, University of Florida – NFREC, Quincy, FL (Post-doctoral fellow) and Matthew Barre, Slant Range, CA; Shep Eubanks, University of Florida, Gadsden County Extension

Amount, source of funds and year/s, and research support: Florida Department of Agriculture and Consumer Services – Specialty Crops Block Grant Program – $229,004 (2017-2019); and Farmer cooperators from Florida Watermelon Association and National Watermelon Association

Background: Conventional field scouting practices typically covers only ~5% of the field at a time, and thus chances of missing early stages of disease foci is high. This can lead to delayed application of critical fungicides, which can affect disease spread, and yield of watermelon and cucumber.

Key research activities: The objectives of the study were 1) Evaluating whether multi-spectral imagery on unmanned aerial vehicles can improve accurate assessment of disease severity compared to conventional scouting approaches and 2) Assessment of Green, Red and Red-Edge Normalized Difference Vegetation Indices (NDVI) and Stress Index for conventional scouted areas in comparison to areas identified with multi-spectral sensors and 3) Identifying abiotic issues in comparison to biotic issues using multi-spectral sensors

Results of research: Multi-spectral sensor assisted scouting had significantly higher assessments of disease severities compared to conventional scouting approach. Green, Red and Red-Edge NDVI were significantly lower in multi-spectral sensor-assisted locations and significantly higher stress index in comparison to conventional scouted areas. Studies on assessments in areas with biotic and abiotic issues are in progress.

Project Title: Improving watermelon production in Florida by developing a field-based recombinase polymerase amplification assay for critical viruses and field management of the insect vectors using Kaolin Clay

Principal Investigator: Dr. Mathews L. Paret, University of Florida - NFREC, Quincy, FL and Plant Pathology Department, Gainesville, FL

Other Project Investigators and collaborators: Dr. Xavier Martini, University of Florida - NFREC, Quincy, FL and Entomology and Nematology Department, Gainesville, FL; Dr. Pamela Roberts, University of Florida – SWFREC, Immokalee, FL and Plant Pathology Department, Gainesville, FL; Dr. Melanie Kalischuk, University of Florida – NFREC, Quincy, FL (Post-doctoral fellow), and Bob Hochmuth, University of Florida – NFREC, Live Oak, FL.

Amount, source of funds and year/s, and research support: Florida Watermelon Association, Georgia Watermelon Association, National Watermelon Association and Gulf Coast Watermelon Association - $22,000 (2018-2019)

Background: Many viruses cause major damage to watermelon and other cucurbits in Florida. However, the molecular (DNA/RNA-based) diagnostic methods currently available can only be used under lab conditions. Some of these viruses are transmitted by white flies which has been a major issue in the recent years and currently there is limited information of the utility of physical protectants like Kaolin clay in reducing white fly populations and virus incidence.

Key research activities: Recombinase Polymerase Assay is a new molecular assay that can be conducted under field conditions. Cucurbit leaf crumple virus (CuLCrV), a single stranded DNA virus of the genus Begomovirus, causes Cucurbit leaf crumple disease in many of the species of the cucurbit family. The study reports the first time development and standardization of an isothermal-based recombinase amplification assay (RPA) for detection and diagnosis of CuLCrV.

Results of research: The RPA assay developed for CuLCrV was specific and did not show cross-amplification to other cucurbit infecting viruses. Detection of CuLCrV in watermelon, wild cucumber and citron was completed by RPA directly on samples with threshold detection time of less than 30 minutes for upto 8 samples. Further progress will include the development of RPA against other common viruses affecting cucurbits, assessing its sensitivity and specificity, and evaluating the assay in commercial watermelon fields in South and North Florida. Studies on the impact of Kaolin clay on white fly populations and disease incidence is in progress.

Project Title: Identification and management of Fusarium wilt races in Florida and the Southeast.

Names of PI and others on the project team: Dr. Nicholas S. Dufault, UF-IFAS, Plant Pathology, Dr. Bimal Amaradasa, UF-IFAS Plant Pathology (Currently at Virginia Tech), Dr. Tatianna Sanchez, Alachua County Horticultural Extension Agent, Dr. Mathews L. Paret, UF-IFAS, North Florida Research and Education Center, Quincy, FL; Dr. Josh Freeman, UF-IFAS, North Florida Research and Education Center, Quincy, FL; Bob Hochmuth, UF-IFAS, North Florida Research and Education Center, Quincy, FL; Anthony Drew (Retired) Levy County Extension Agent; Dr. Pingsheng Yi, Department of Plant Pathology, University of Georgia, Dr. Anthony Keinath, Clemson University.

Amount and sources of funds: USDA SCRI Grant (2015 to 2018), FDACS Specialty Crop Block Grant (2018-2020), National Watermelon Association (2017), Georgia Watermelon Association (2018).

Background. Fusarium wilt continues to be a problem for watermelon producers in the Southeast and throughout the U.S. The disease used to be managed with long rotations (> 5 years) away from a host crop, however recently the disease has been a problem in fields with a rotation of 7 years or more. Resistance is available for this pathogen, but it is primarily for race 0 and 1. Other chemical methods are available, but the results are variable in disease control and can be expensive, especially for small acreage (< 30 acres) producers. Improving the integrated management of this pest starts by learning more about the population of the pathogen present and developing new tools for disease management.

Brief description of key research activities:

- Collected over 100 isolates of Fusarium oxysporum from 10 counties in Florida and have stored them for long term at the University of Florida
- Performed molecular, physiological and race typing assays, to characterize 30 plus isolates; including strains identified as Race 3 in Florida.
- Established coordinated field trials at the NFREC in Live Oak and PSREU in Citra to assess impact of disease on watermelon yield and response to several fungicides and planting dates.
- Developing novel integrated management tools (e.g. varied planting date, bioassay for pathogen detection and climate web tool) to improve the management this disease while relating it to other research being conducted on watermelon grafting and fumigation.

Status/Results of research results to date:

- Physiological testing indicated that various isolate groups responded uniquely to constant and oscillating temperatures with optimal mycelial growth occurring around 28 C (83 F).
- Molecular assays showed there was no geographical relationship between isolates which indicates that pathogen evolution is location independent. This means that Florida's watermelon regional management strategies are not likely selecting for pathogen changes and some other unknown factors are important for isolate divergence.
- Race 3 has been officially confirmed in Florida (https://doi.org/10.1094/PDIS-10-17-1649-PDN) and was observed in Lee, Marion and Madison Counties.
- Watermelon field trials have indicated that varying planting date can affect Fusarium wilt incidence, but 2018 created a unique situation that limited these affects.
- Proline® (prothioconazole) does provide disease control, however, results were variable and not reliable over 3 years.
- Current research is being done to complete the development of a bioassay for Fusarium will detection which will be coupled with a climate risk model being planned to release in 2020 on Agroclimate (http://agroclimate.org/).

Project Title: Determining the efficacy of pre-plant fumigation and fungicide applicatin for Fusarium wilt management in seedless watermelon.

Principal Investigator: Josh Freeman. Associate Professor of Horticulture. University of Florida, North Florida Research and Education Center. Email: joshuafr@ufl.edu. Phone: 850-875-7128.

Amount, source of funds and year/s, and equipment or research contributions: Alabama Watermelon Association (now the Gulf Coast Watermelon Association) - $6,149 (2018); National Watermelon Association - $14,170 (2017).

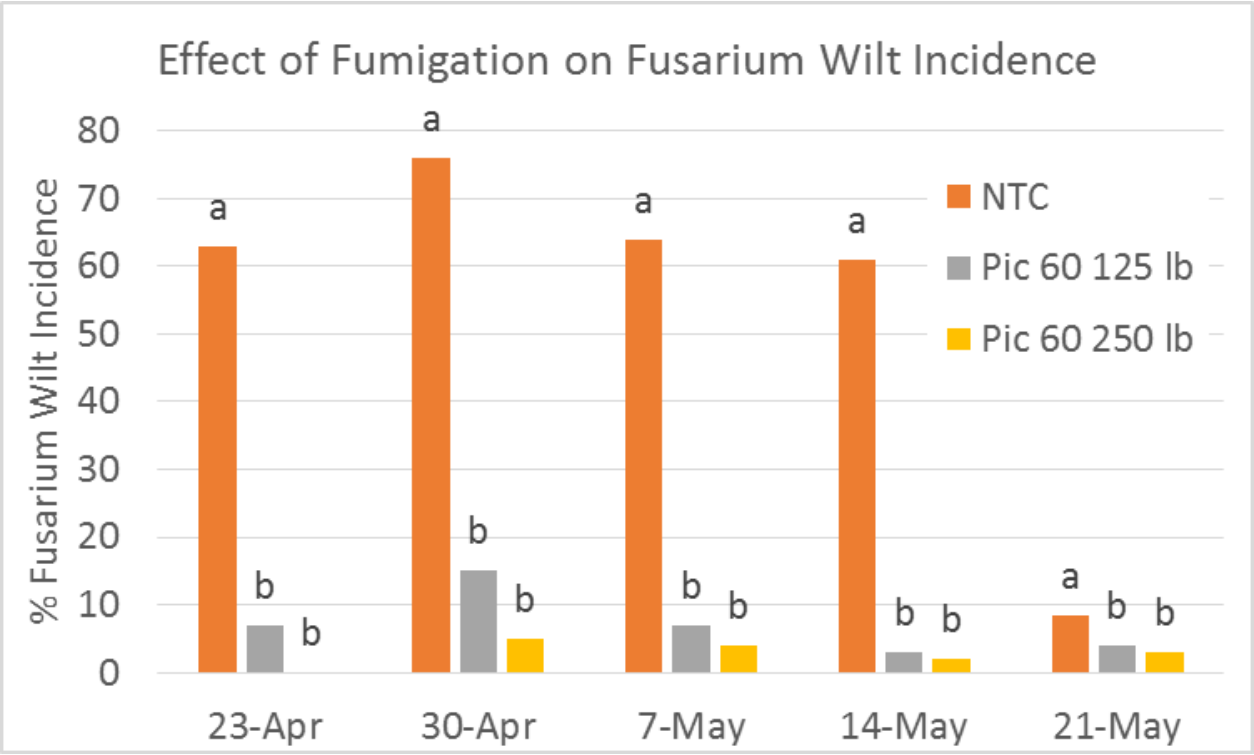
Project Summary: This project included three fumigation trials which investigated the effect of a chloropicrin and telone mixture (Pic Clor 60) using highly retentive plastic mulch on Fusarium wilt of watermelon. The project was intended to investigate the impact of reduced bed geometry, narrow and taller plasticulture beds, but the equipment did not function properly so this treatment was not implemented. Highly retentive plastic films were used because they have been shown to reduce fumigant use by as much as 50% while maintaining efficacy. The first trial investigated a low and high rate of Pic Clor 60 compared to a non-treated control while a second trial investigated the effect of prothioconazole (Proline) with and without the use of Pic Clor 60 on Fusarium wilt incidence, also compared to a non-treated control. A third trial was implemented to demonstrate the benefit of highly retentive plastic mulch.

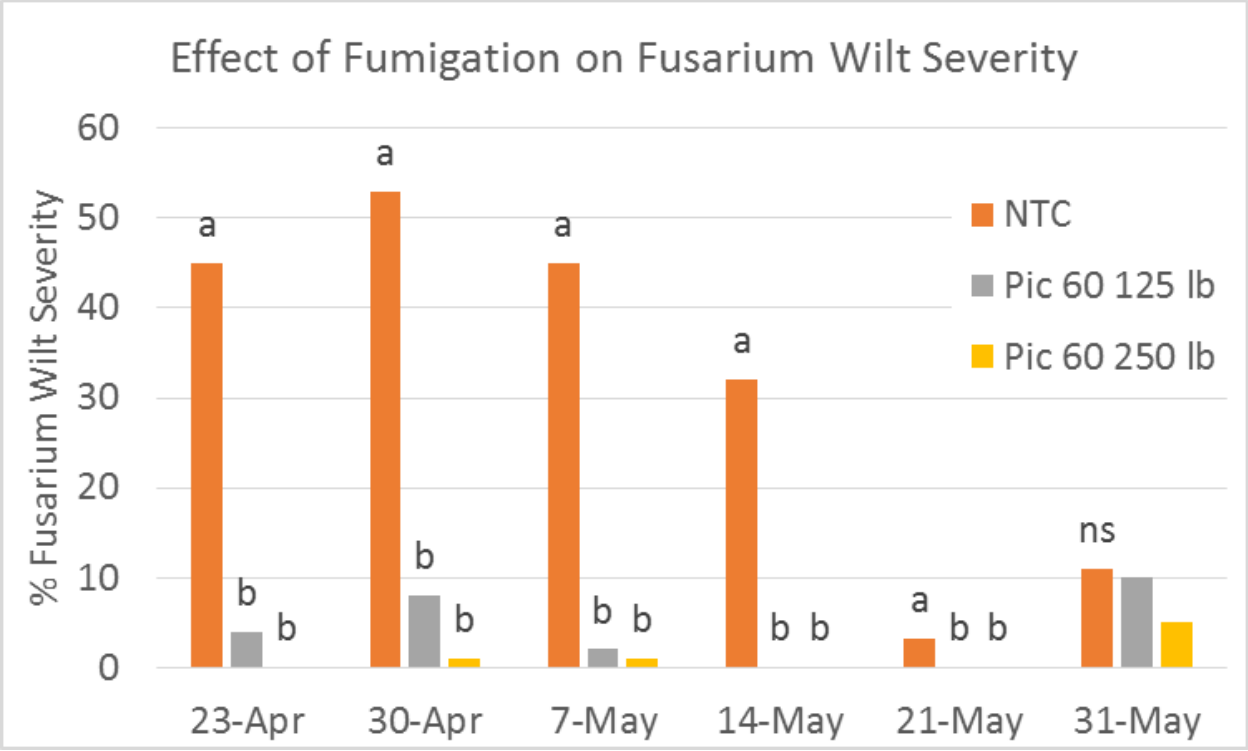
Experimental Methods: This research was conducted on a grower's farm in Jackson County Florida in a field with prior incidence of Fusarium wilt. Fumigant was applied on March 13 and seedlings were transplanted on March 29. Trials and treatments are listed in a table below. Beds were 8" tall and 30" wide and all replicated treatments were covered with 1.25 mil black Berry Totally Impermeable Film (TIF). In the first experiment, plots consisted of 5 rows 100' long and contained 100 plants. Treatments were replicated three times. Disease incidence and severity was rated on a weekly basis at the onset of symptoms and plots were harvested three times. A second trial was established in the same field that examined the use of Proline with and without Pic Clor 60. Proline was injected through the drip line at 5.7 oz/a at planting followed by two additional applications at 14 day intervals. Experimental plots consisted of a single row 75' long and contained 15 plants. Treatments were replicated 5 times. Disease measurements were conducted in a similar manner but harvest data was not taken. A demonstration trial was established where 250 lb/acre of Pic Clor 60 was applied under either 1.25 mil Berry TIF or 1.25 mil Berry standard low density film (LDPE). Each treatment consisted of a single row 300' long containing 100 plants. Disease measurements were conducted in a similar manner but harvest data was not taken. The seedless variety Troubadour along with SP-6 pollenizer was used in the entire field.

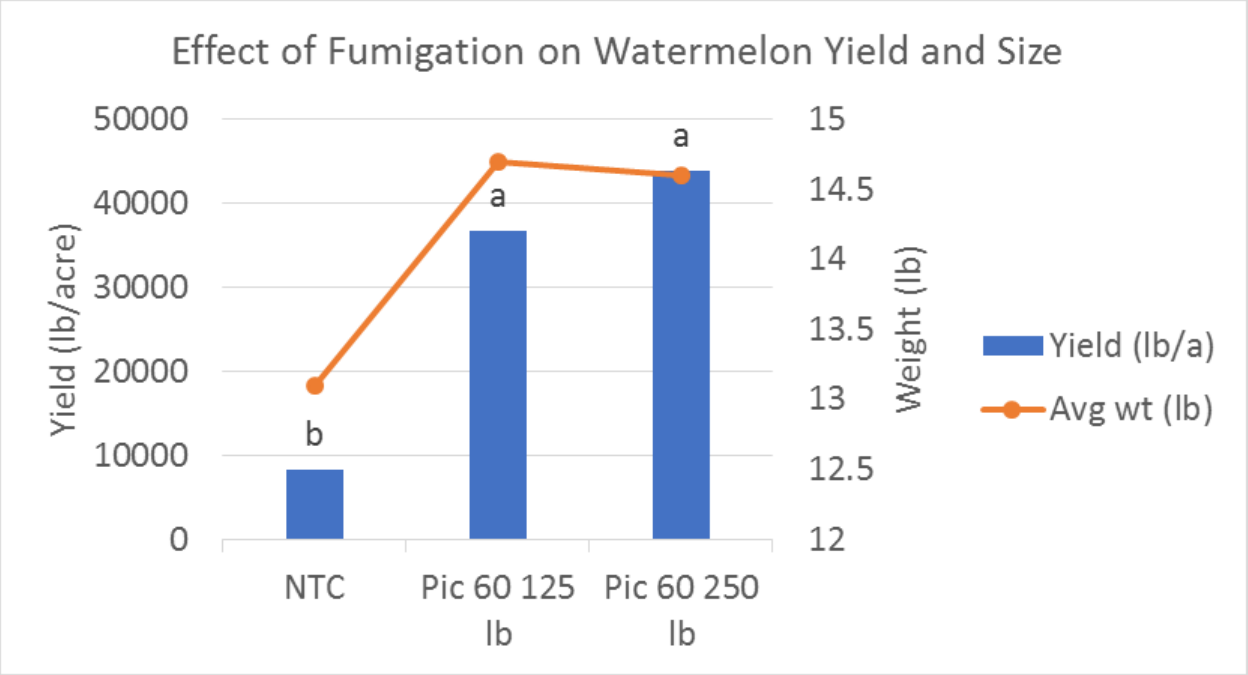
Summary of Findings: Fusarium wilt incidence across all three trials was very high, in many cases resulting in complete mortality in the non-treated plots. Data from all three experiments produced very good results and the fumigation treatments significantly reduced Fusarium wilt incidence and increased fruit yield. Gummy stem blight moved into the field close to harvest and likely reduced total yield but the yield results were still very clear. Data from the first trial did not indicate a significant difference between Pic Clor 60 rates for disease or yield but the higher rate consistently exhibited less disease and produced greater yield. Both fumigant rates resulted in significantly less Fusarium wilt than the control. The second trial indicated a clear reduction in Fusarium wilt in plots treated with Pic Clor 60 or Pic Clor 60 plus Proline compared to other treatments. There was no difference between the Pic Clor 60 and Pic Clor 60 plus Proline treatments. When Proline was used without Pic Clor 60, Fusarium wilt was not statistically reduced compared to the non-treated control and disease incidence was still very high, often greater than 50% incidence. The demonstration trial, while not statistically analyzed, also produced very good results which further confirm the benefits of TIF. Fusarium wilt incidence, and nutsedge populations, were much lower in the bed covered with TIF compared to LDPE.
Though this was only one year of experimentation the results were convincing and indicate the potential of managing Fusarium wilt of watermelon with available fumigant tools. It is our opinion that the size of the plasticulture bed is very

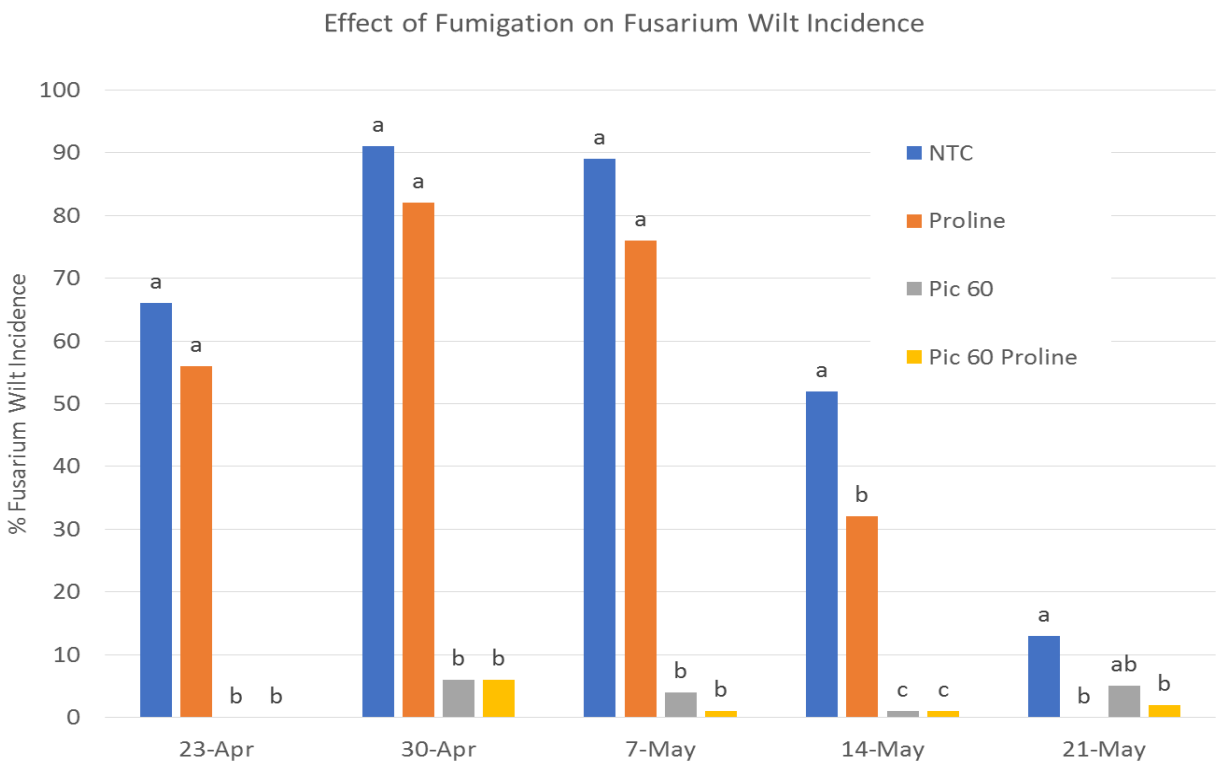
important. Bed size contributes to fumigant retention and results in a greater volume of treated soil which allows susceptible watermelon roots to remain in treated soil for a longer period. It is unclear if these results would be achievable with short narrow beds. This experimentation only investigated a single fumigant formulation, Pic Clor 60, which is 60% chloropicrin. Chloropicrin is known to have good activity against soil-borne fungi and is likely contributing most to the efficacy on Fusarium. The Telone portion of the mixture (40%) will provide activity against nematodes and the mixture of the two fumigants has been proved to have good activity on nutsedge, especially when used under TIF. Using a mixture with less chloropicrin may not provide results similar to these. Further experimentation will be conducted in 2019 to confirm these results and investigate other fumigant mixtures. It is also clear that the use of highly retentive TIF is critical for maximizing fumigation efficacy. The producer that hosted this research had not used TIF before. After viewing the field and the difference between LDPE and TIF he is transitioning to TIF for all of his soil fumigation.

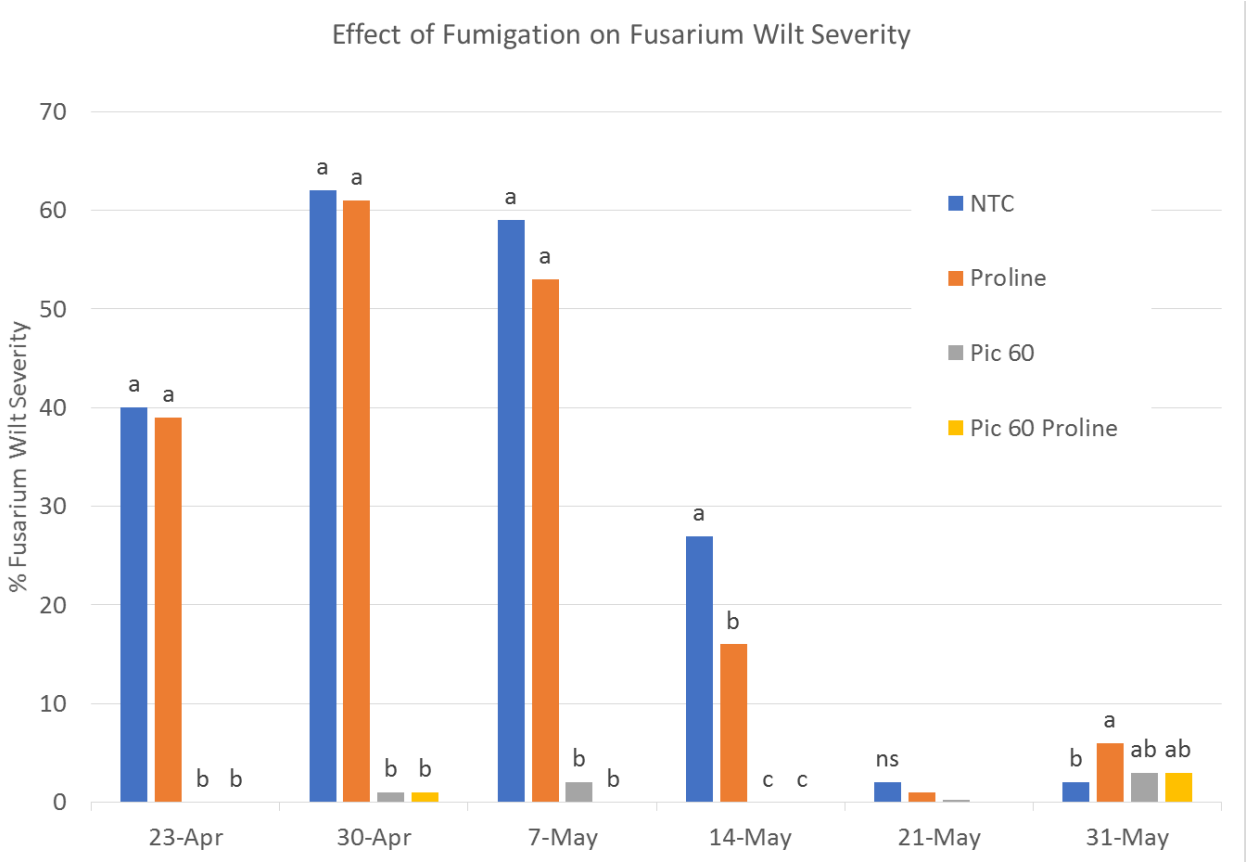
2018 Fusarium Wilt Fumigation Treatments		
Pic Clor 60 Rate	**Pic Clor 60 + Proline**	**Pic Clor 60 TIF vs LDPE**
Non-treated control	Non-treated control	250 lb/acre Pic Clor 60 TIF
125 lb/acre Pic Clor 60 TIF	333 lb/acre Pic Clor 60	250 lb/acre Pic Clor 60 LDPE
250 lb/acre Pic Clor 60 TIF	333 lb/acre Pic Clor 60 + Proline	
	Proline	




Effect of Fumigation on Fusarium Wilt Severity
% Fusarium Wilt Severity
60
50
40
30
20
10
0
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
ns
23-Apr
30-Apr
7-May
14-May
21-May
31-May
NTC
Pic 60 125 lb
Pic 60 250 lb


Effect of Fumigation on Watermelon Yield and Size
Yield (lb/acre)
50000
40000
30000
20000
10000
0
Weight (lb)
15
14.5
14
13.5
13
12.5
12
b
a
a
NTC
Pic 60 125 lb
Pic 60 250 lb
Yield (lb/a)
Avg wt (lb)


Effect of Fumigation on Fusarium Wilt Incidence
% Fusarium Wilt Incidence
100
90
80
70
60
50
40
30
20
10
0
23-Apr
30-Apr
7-May
14-May
21-May
NTC
Proline
Pic 60
Pic 60 Proline
a a b b
a a b b
a a b b
a b c c
a b ab b


Effect of Fumigation on Fusarium Wilt Severity
% Fusarium Wilt Severity
70
60
50
40
30
20
10
0
23-Apr
30-Apr
7-May
14-May
21-May
31-May
NTC
Proline
Pic 60
Pic 60 Proline
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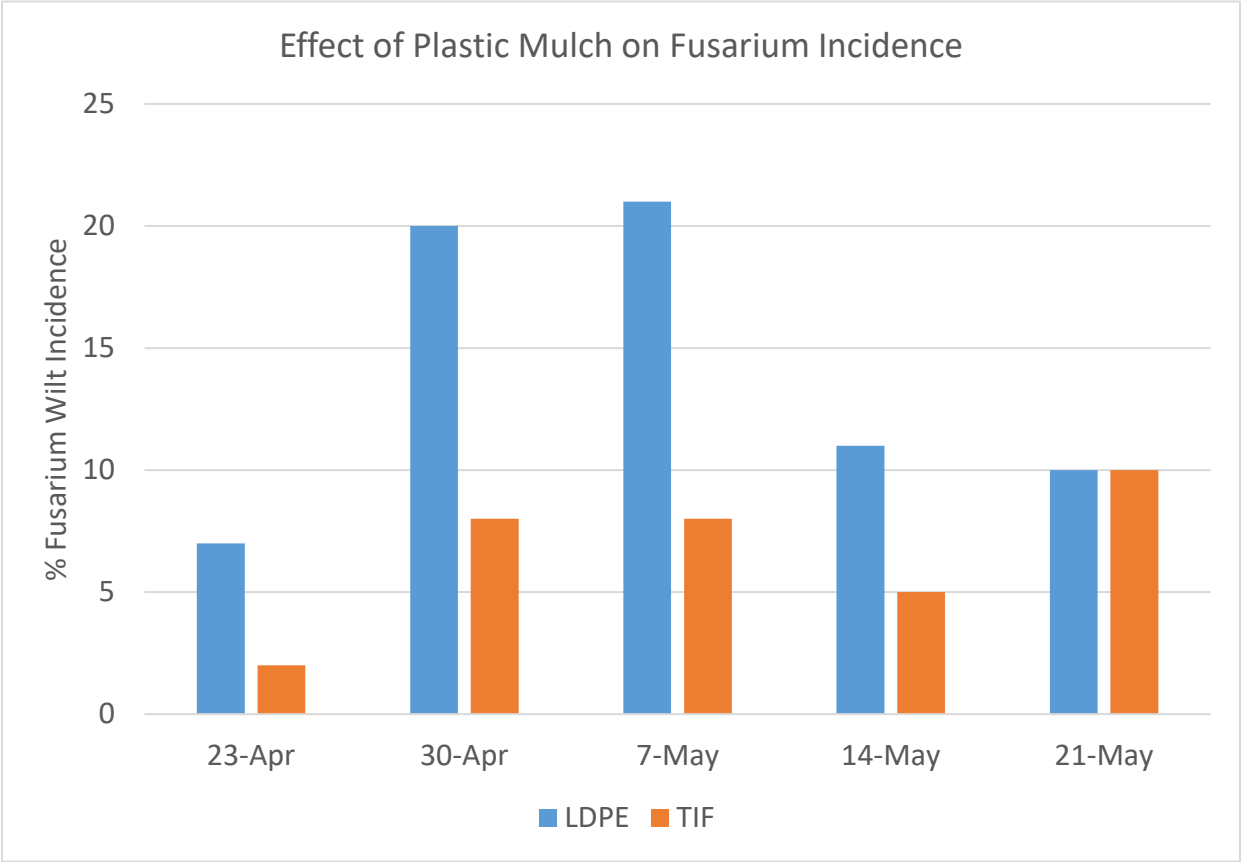
a a b b
a a b b
a b c c
ns
b a ab ab


Effect of Plastic Mulch on Fusarium Incidence
% Fusarium Wilt Incidence
25
20
15
10
5
0
23-Apr
30-Apr
7-May
14-May
21-May
LDPE
TIF