Optimization of Preventative Biorational Strawberry Fruit and Root Disease Management Techniques

Progress report for LNE20-401

Project Type: Research and Education

Funds awarded in 2020: $244,349.00

Projected End Date: 04/30/2023

Grant Recipients: West Virginia University; Virginia Polytechnic Institute and State University; University of Maine

Region: Northeast

State: West Virginia

Project Leader:

Dr. Mahfuz Rahman
West Virginia University

Project Information

Performance Target:

Two major plant propagators save fungicide cost and strawberry losses from anthracnose worth $100,000. 150 strawberry growers adopt alternative fumigation methods on 300 acres of fruit production field to improve yield, generating $150,000 additional annual income.

Introduction:

Problem and Justification: In the Northeast and Mid-Atlantic, there is a growing concern related to latent strawberry diseases, specifically Anthracnose Fruit Rot (AFR). AFR causes a latent infection on strawberry foliage, creating problems for both propagators and fruit producers who believed they had disease free plant material. Currently there is no reliable diagnostic method-based protocol for plant propagators to test for anthracnose and make marketing decision or alert fruit producers of a need for preventative measures. As a result, fruit producers are caught off-guard due to symptom appearance during fruit set affecting marketable fruit. There is growers identified need to detect latent infection on cuttings and plug plants to prevent occurrence of anthracnose. Black root rot complex (BRRC) and crown rot (CR) caused by multiple soil-borne fungi that cause plant mortality or severely affect plant vigor and productivity of strawberries are also limiting factors after methyl bromide had been phased out. These diseases are often more problematic for small-scale growers who grow strawberries organically and/or utilize a perennial matted row system with limited option for crop rotation, which is a common practice in the Northeast.

Solution and Approach: A newly developed latent infection diagnostic method (combination of herbicide-based bioassay and DNA-based PCR method) will be utilized in collaboration with plant propagators to detect latent infection from tips

and plug plants to predict anthracnose occurrence, and to recommend potential preventative measures. Results from the application of probiotic organisms on strawberry plugs prior to planting from our initial work suggest that early colonization of plug root systems by plant growth promoting microbes competitively exclude colonization of roots/crowns by pathogens in field setting to reduce plant mortality in a perennial system. This practice, along with mustard bio-fumigation, anaerobic soil disinfestation were also found to be a comprehensive approach to manage disease pressure sustainably and economically.

Milestones and Performance Target: In the proposed work, we will capitalize on our initial findings to educate strawberry growers on sustainable options for managing major root and crown diseases in multiple states. In addition, we will develop a diagnostic-based framework to support plant suppliers to produce latent infection free transplants. These plant suppliers have a wide customer base (fruit producers) in the Northeast and Mid-Atlantic USA. Two Fruit producers from each of WV, ME and VA will evaluate treated and non-treated plants on their farms in an experimental setting by planting either in bio-fumigated or anaerobically disinfested soil or non-treated plots.

Two major plant propagators save fungicide cost and strawberry losses from anthracnose worth $100,000. 150 strawberry growers adopt alternative fumigation methods on 300 acres of fruit production field to improve yield, generating $150,000 additional annual income.

Cooperators

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Research

Hypothesis:

1) Diagnosis of latent infection on foliage of strawberry propagation materials will prevent entry of infected plants into the fruiting field or alert growers about preventative measures.
2) Pre-colonization of strawberry plug plant roots by beneficial microbes impedes fungal infections associated with BRRC and crown rot.
3) Microbiological assessment of root system and fruit analysis for bioactive compounds unravel the mechanism of BRRC suppression and growth and quality enhancement, respectively.
4) Probiotic microbes and bio-fumigation improves availability of soil nutrients to strawberry plants, enhancing plant vigor and productivity.

Materials and methods:

Objective 1. Strawberry cuttings and plug plant samples will be collected from two plant propagation facilities and evaluated for latent infections by the method better described as bio-PCR. Briefly, foliar samples will be subjected to gramoxone protocol to induce tissue senescence and organism start to multiply when incubated in high humidity. Within 72 hours of incubation, required amount of tissues will be collected for PCR regardless of any visible growth. For specific detection of the anthracnose fruit rot fungus, PCR primers designed to amplify the fungal MAT1-2 gene will be used. The subsequent micro tube hybridization (MTH) will be done using the PCR products as probes to replace the laborious electrophoresis step providing us sequence information and high-throughput screening as described by Furuta et al. (2017). A schematic diagram modified from Furuta et al. included in other relevant research information. In general, a targeted sampling will be done based on visibility of black spots on leaves of cuttings and plug plants. Anthracnose infections on foliage in some cases produce black spots. However, other fungi may also cause black spots on a leaf. As part of our educational program, we will educate beneficiaries how to separate these symptoms. Five cuttings will be randomly collected from each box of 1000 tips supply for latent infection evaluation. Cuttings/tips that are set on plug trays by plant propagators will be tracked by a unique tracking system to relate to samples taken. If cuttings from a box are found positive for latent infection by AFR causing fungus, plug plants from those cuttings will be sampled in higher proportion (2%) and conduct the diagnostic method again. This will enable us to understand how infection spreads under sprinkler irrigation in a plug production facility. This will also be useful to suggest plant propagators on making decision whether to cull those plants or sell to fruit producers with a written instruction how to handle those plants and if any preventative spray schedule will have to be followed. We will send lab analyses results to plant propagators with best recommendations to be conveyed to the fruit producers. Plug plant producers will contact their cuttings/tips suppliers with the results and consult how to keep the clean plant supply chain active. PI of the project will take trips to talk to tips suppliers' and to suggest any changes needed of the production facility and practices. Results will be compiled as the efficacy of the modified protocol in tracing latent infection.

Objective 2.

Treatments: This study will be conducted in two phases; the first phase will be at the plug plant production facility of the prime cooperator Mr. Reuben Martin (Shippensburg, PA). The second phase of the research will be conducted at the

fruiting fields of selected cooperators. Tip immersion in probiotic bacteria TerraGrow will be done prior to Inoculation of 'Johnny's 512 Organic Mix' @ 1oz/100 gallons with or without pasteurization. A total of 900 strawberry plug plants of CV Chandler will be grown on pasteurized+treated and 500 on nonpasteurized+treated planting mix. A total of 1200 plug plants will be grown on regular mix without treatment destined for the non-treated control plot, bio-fumigated or ASD plots in fruiting fields. Growers' standard in the Northeast is possible rotation but no synthetic fumigant use.

*Fruiting field trial: We will conduct six on-farm trials (two in each of WV, VA and ME; please see support letters from grower cooperators) with six different treatments that will include: 1) plug plants grown on pasteurized planting mix treated with TerraGrow and planted in non-treated fruiting field plots (Effect of TerraGrow with higher colonization potential); 2) plug plants grown on non-pasteurized planting mix treated with TerraGrow and planted in ASD fruiting field plots (Combined effect of TerraGrow and ASD); 3) plug plants grown on non-treated planting mix and planted in ASD plots (Effect of ASD); 4) plug plants grown on non-pasteurized planting mix treated with TerraGrow and planted in non-treated fruiting field plots (Effect of TerraGrow with easier treatment option); 5) plug plants grown on non-treated planting mix and planted in 'Caliente 199' mustard cover crop incorporated plots (Effect of bio-fumigation); 6) plug plants grown on non-treated planting mix and planted in nontreated fruiting field soil as non-treated check. Treatments will be replicated four times in a randomized complete block design with each replicate plot consisting of a 20 ft long and 5 ft wide plastic mulched drip irrigated raised bed. The black root rot-susceptible but popular strawberry cultivar Chandler will be used in the study. Each plot will have 20 plants in two staggered rows on a bed spaced 1 ft apart row to row and 2 feet plant to plant (see detailed pictorial plot plan in relevant research information).

1. Method for mustard cover crop and ASD based bio-fumigation: 1. Fruiting field plots will be prepared at the grower cooperators' sites in a randomized complete block design that accommodates all six treatments mentioned above. Plots that will receive mustard bio-fumigation and ASD treatments will be prepared accordingly to match the projected planting date of Aug 15 – Sept 7, 2020. In case there is not enough moisture in the soil, irrigation will be provided to bring soil moisture to field capacity and covered with plastic at the same time while preparing the whole bed. This whole process will be completed at least 21 days before planting. 2. ASD on selected plots will be done in 3 steps as mentioned in the literature review section according to Shennan et al. (2017).

Data collection and statistical analyses. We will collect total fruit yield and the incidence of BRRC symptomatic plants as well as any wilted plants from all treatments. Severity of black root rot will be measured on a 0-5 scale where 0 = no disease symptom; 1 = 1 to 5% plants are stunted; 2 = 6 to 10% plants are stunted; 3 = 11 to 25% plants are stunted; 4 = 26 to 50% plants are stunted; 5 = 51 to 100% of plants are stunted and some wilting of the plants. The disease index for each replicate plot will be calculated from the number of plants with symptoms and recorded severity on each plant. As the black root rot severity data will be collected on an ordinal scale rather than continuous, a non-parametric data analysis will be done by PROC RANK in SAS (SAS institute, Cary NC). However, yield, nutrient, nematode count and microbial data will be subjected to ANOVA to determine differences of means in various treatments. A linear mixed model will be used where treatment will be considered as a fixed effect, and block as a random effect. Percent data will be transformed using angular transformation (arcsine of square-

rooted value) prior to the analysis. Means will be compared for significant differences by Fisher's protected LSD test (P = 0.05).

-Nutrient and microbiological analyses: Rhizosphere soil will be collected from each plot to analyze available P, K and organic C, lesion nematodes pre-plant and after fruit harvest.

Our initial work on objective 2 indicated that all treatments improved yield and reduced plant mortality. However, we need additional data to determine the best treatment by conducting an economic analysis. Photos of treated plots compared with non-treated control included in other relevant research information.

Research results and discussion:

SARE-2020-smpling-guide One on-farm trial has been set during the fall of 2020. Data will be available in the spring of 2021.

Research conclusions:

N/A

Participation Summary

1 Farmer participating in research

Education

Educational approach:

Project PI and Co-Pis arranged a conference call with Gregg Gordon, President of Aaron's Creek Farm on July 7, 2020 to discuss the importance of testing strawberry propagation materials (tips and plug plants). We discussed in detail the process involved with collecting good sample and evaluating latent infection, and importance of the process. Due to this educational approach, he got clear understanding of sample collection and sending to the plant pathology diagnostic lab of the Co-PI of this project. As we were not able to be at the Aaron's Creek farm in person due to pandemic situation, we also created an educational material illustrating how to take sample and how to conduct the evaluation process to share with the clientele. Right samples were taken and sent to the labs indicating that our educational approach made impact despite unusual situation. We also had similar educational event with Mr. Reuben Martin of Maplewood farm market (Shippensburg, PA). As this grower cooperator do not use internet or email, we had to have the session by teleconferencing. IPM associate, Whitney Dudding later delivered the printed copy of the educational materials (uploaded) to Mr. Reuben Martin. In addition, PI had conversations with Mr. Martin 10 times during the year 2020 to convey educational information related to disease problems in plug plant production system and selling those plants to fruit producers. More specifically, what practices will need to be followed by fruit producers as it relates to the plant health status revealed by diagnostic assays for latent infections. These educational conversations also included a new disease problem known as 'Pestalotiopsis blight' caused by Neopestalotiopsis sp.

Milestones

Milestone #1

What beneficiaries do and learn:

Strawberry growers across 3 states receive online and printed version of the information and survey about the project to learn new opportunities to buy latent infection free transplants. They also learn about new biorational treatments to manage soil-borne pathogens in strawberry production systems. Interested ones respond to the survey.

Proposed number of farmer beneficiaries who will participate:

250

Proposed number of agriculture service provider beneficiaries who will participate:

3

Actual number of farmer beneficiaries who participated:

29

Actual number of agriculture service provider beneficiaries who participated:

3

Proposed Completion Date:

April 30, 2020

Status:

In Progress

Accomplishments:

In March 2020, PI had several zoom meetings with the Co-PIs of the project to discuss and strategize what's the best way to recruit beneficiaries during a pandemic situation. After a thorough discussion, we decided to send a qualtrix survey to the predominantly organic and small strawberry growers in multiple states (WV, MD, VA, PA, ME). A copy of the survey can be found here: Final-Strawberry-Producer-Survey-3. As many of our target beneficiaries do not use online platform, it was a little difficult to get very good response. We did not send printed survey to the potential beneficiaries yet as we are most interested to get these to the growers in meetings once the pandemic situation is over. However, our efforts ended up recruiting 29 beneficiaries. It is note-worthy that 15 out of 29 beneficiaries showed interest in becoming cooperators by establishing trials in their farms. Recruitment of beneficiaries will be continued during face to face meetings once the pandemic situation is over. We are also in the process of revising the survey and considering sending print copies by postal mail depending on the future situation.

Milestone #2

What beneficiaries do and learn:

Beneficiaries help PI and Co-PIs to collect cutting samples and bare root plants from Maplewood Farm Market and Aaron's Creek Farms to complete diagnostics

for latent infection incidence and severity

Proposed number of farmer beneficiaries who will participate:

2

Actual number of farmer beneficiaries who participated:

2

Proposed Completion Date:

July 15, 2020

Status:

Completed

Date Completed:

October 15, 2020

Accomplishments:

Project PI and Co-Pis arranged a conference call with Gregg Gordon, President of Aaron's Creek Farm on July 7, 2020 about the importance of testing strawberry propagation materials (tips and plug plants). We discussed in detail the process involved with collecting good sample and evaluation of latent infection and importance of the process. Due to this educational approach, he got clear understanding of sample collection and sending to the plant pathology diagnostic lab of the Co-PI of this project. As we were not able to be at the Aaron's Creek farm in person due to pandemic situation, we also created an educational material illustrating how to take sample and how to conduct the evaluation process to share with the clientele. Right samples were taken and sent to the labs indicating that our educational approach made impact despite unusual situation. We also had similar educational event with Mr. Reuben Martin of Maplewood farm market (Shippensburg, PA). As this grower cooperator do not use internet or email, we had to have the session by teleconferencing. IPM associate, Whitney Dudding later delivered the printed copy of the educational materials (uploaded) to Mr. Reuben Martin. In addition, PI had conversations with Mr. Martin 10 times during the year 2020 to convey educational information related to disease problems in plug plant production system and selling those plants to fruit producers. More specifically, what practices will need to be followed by fruit producers as it relates to the plant health status revealed by diagnostic assays for latent infections. These educational conversations also included a new disease problem known as 'Pestalotiopsis blight' caused by *Neopestalotiopsis* sp.

Milestone #3

What beneficiaries do and learn:

Beneficiaries receive diagnostic results and follow recommendations whether to cull infected plants or go ahead with plug setting as no problem found

Proposed number of farmer beneficiaries who will participate:

2

Actual number of farmer beneficiaries who participated:

2

Proposed Completion Date:

July 31, 2020

Status:

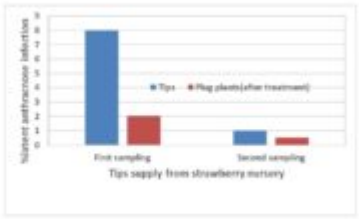
Completed

Date Completed:

October 15, 2020

Accomplishments:

Project personnel with vast technical knowledge evaluated the samples and results were conveyed to both plug plant producers that helped them taking the right decision about either spraying fungicides or discarding problematic plants. In addition, they could communicate with their tips suppliers to prevent supply of latently infected plants. Both of the cooperators have now gained knowledge on the latent infection cycle and willing to work with plant suppliers to secure infection-free plants.



Detection of latent infection on strawberry foliage

Strawberry tips from first sampling had 8% incidence of latent anthracnose infection as revealed by our lab diagnosis results. Second sampling showed 2% infection incidence. Due to conveying the results to the grower cooperator and adoption of suggested mitigation measures infection incidence was reduced to 1% and 0.5%, respectively.

Milestone #4

What beneficiaries do and learn:

Beneficiaries with technical support from PI and co-PI collect suspected plug plant sample for diagnostic evaluation

Proposed number of farmer beneficiaries who will participate:

2

Actual number of farmer beneficiaries who participated:

2

Proposed Completion Date:

September 15, 2020

Status:

Completed

Date Completed:

October 15, 2020

Accomplishments:

Beneficiaries with technical support from PI and co-PI collected suspected plug plant sample for diagnostic evaluation and sent to the diagnostic labs.

Milestone #5

What beneficiaries do and learn:

Beneficiaries set demonstration trials in their farms following treatment application guidelines from project personnel

Proposed number of farmer beneficiaries who will participate:

6

Proposed number of agriculture service provider beneficiaries who will participate:

3

Actual number of farmer beneficiaries who participated:

1

Proposed Completion Date:

September 30, 2020

Status:

In Progress

Accomplishments:

Due to the pandemic situation, it was difficult to grow treated plants and send to the beneficiaries. However, one demonstration was established in WV.

Milestone #6

What beneficiaries do and learn:

Strawberry growers attend winter meetings and trainings, lean more about strawberry disease management through project activities like latent infection free plants and biorational treatments of fields

Proposed number of farmer beneficiaries who will participate:

250

Proposed number of agriculture service provider beneficiaries who will participate:

6

Actual number of farmer beneficiaries who participated:

1

Actual number of agriculture service provider beneficiaries who participated:

1

Proposed Completion Date:

March 31, 2021

Status:

In Progress

Accomplishments:

will be updated next year

Milestone #7

What beneficiaries do and learn:

Strawberry growers attend field demonstration days, witness the efficacy of alternative fumigation technologies and learn the techniques from the project staff and fellow growers

Proposed number of farmer beneficiaries who will participate:

180

Proposed number of agriculture service provider beneficiaries who will participate:

6

Actual number of farmer beneficiaries who participated:

1

Actual number of agriculture service provider beneficiaries who participated:

1

Proposed Completion Date:

July 10, 2021

Status:

In Progress

Accomplishments:

will be updated

Milestone #8

What beneficiaries do and learn:

Strawberry growers participate in similar activities for one more year and adopt new practices

Proposed number of farmer beneficiaries who will participate:

150

Proposed number of agriculture service provider beneficiaries who will participate:

1

Actual number of farmer beneficiaries who participated:

1

Actual number of agriculture service provider beneficiaries who participated:

1

Proposed Completion Date:

August 31, 2022

Status:

In Progress

Accomplishments:

will be updated

Milestone Activities and Participation Summary

EDUCATIONAL ACTIVITIES:

11 Consultations

1 Curricula, factsheets or educational tools

2 Webinars / talks / presentations

Learning Outcomes

2 Farmers reported changes in knowledge, attitudes, skills and/or awareness as a result of their participation

3 Agricultural service providers reported changes in knowledge, skills, and/or attitudes as a result of their participation

Key areas in which farmers reported changes in knowledge, attitude, skills and/or awareness:

Not updated yet

Performance Target Outcomes

TARGET #1

Target: number of farmers:

2

Target: change/adoption:

Two major plant propagators save fungicide cost and strawberry losses from anthracnose worth $100,000

Target: amount of production affected:

One hundred acres

Target: quantified benefit(s):

$100,000

TARGET #2

Target: number of farmers:

150

Target: change/adoption:

150 strawberry growers adopt alternative fumigation methods on 300 acres of fruit production field to improve yield, generating $150,000 additional annual income.

Target: amount of production affected:

Three hundred acres

Target: quantified benefit(s):

$150,000

Information Products

- Strawberry sample collection guide and latent infection diagnosis (Manual/Guide)