CURRENT METHODS OF THE NATIONAL COTTONSEED TREATMENT PROGRAM AND PROPOSED CHANGES IN PROTOCOL

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Abstract

There are several pathogen isolation and/or quantification methods included in the protocol of the National Cottonseed Treatment program. Currently cooperators use a randomized experimental block design to evaluate the fungicide treatments that have been nominated for the designated growing season. Roughly 30 days after planting, cooperators collect 100 random seedlings from each location's nontreated check plots and send them to the West Tennessee Research and Education Center for processing and evaluation. Those seedlings are split into 2 separate groups which undergo disease rating, node counts, and separate sterilization/pathogen isolation methods. According to which group they are in, seedling roots are plated on a series of selective media in an effort to quantify the pathogen populations of *Thielaviopsis basicola* and *Pythium* spp. In addition to the plating of roots on selective media; toothpick baiting, PDA, and soil dilution plating using selective media are also utilized to further assess the pathogen populations of *Rhizoctonia solani, Fusarium* sp., *Thielaviopsis basicola*, and *Pythium* spp., respectively. Although the current protocol has yielded extremely useful data over the years, there are more current and updated methods for isolation and quantification of pathogens that would enhance the National Cottonseed Treatment program. In an effort to make the sampling process more efficient, modern, and reliable, the removal of some current methods, the use of genetic confirmation, and the use of new selective media will be discussed.

Introduction

The National Cottonseed Treatment trial has been conducted over the past 23 years, and has continued to provide data on soil borne pathogens of cotton seedlings in an effort to analyze seed treatment efficacy. There are usually 12-20 cooperators that put in field trial locations with varying environmental circumstances across the United States cotton belt. Each year, chemical industry representatives nominate seed treatments to test their effectiveness against soil borne pathogens. Each cooperator is responsible for sending in 100 seedlings which then undergo disease

ratings, plating on selective medias, and soil dilution platings in an effort to not only evaluate the seed treatment efficacy of each nominated treatment, but also analyze populations of *Thielaviopsis basicola*, *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp. in each field trial location. By inheriting and working with this trial this past growing season, the research team at the West Tennessee Research and Education Center has decided to change some of the current methods of the protocol in an effort to make the seedling assessment process more efficient and the data produced from this trial more reliable.

Materials and Methods

All field trial locations are setup using a randomized complete block design, with the rows typically being 20ft long or greater, with 4 to 8 replicates, and the seeding rate specific to location. All seeds have Gaucho 600 applied to them to mitigate any insect damage that may occur during the duration of the trial. Seed treatments are nominated annually, and 3 controls are always utilized: EverGol Prime, Allegiance, and a non-treated check. Thirty days after planting, 100 seedlings and soil samples are taken from the non-treated check plots for each field location. After the seedlings are collected they are sent to the plant health diagnostics lab at the West Tennessee Research and Education Center. Seedlings and soil samples are evaluated through a series of node counts, disease severity ratings, plating onto selective media, soil baiting, and soil dilution plating.

Seedling Assessment

Out of the 100 seedlings received, 5 are randomly selected for node counts. The group of 100 seedlings is split randomly into 2 groups of 50. The first group of seedlings is rated for disease severity in both hypocotyl and root regions. The hypocotyl disease severity rating scale consists of the following: 1= no symptoms, 2= few pinpoint lesions, 3=distinct necrotic lesions, 4= girdling lesion, 5= seedling is dead. Whereas, the root system disease severity rating scale consists of the following: 1= no symptoms, 2= 1-10% of root system discolored, 3= 11-25% of root system discolored, 4= 26-50% of root system discolored, 5= 51-75% of root system discolored, 6= >75% of root system discolored. After being rated for disease severity, the first group of seedlings are surface disinfected, and plated onto antibiotic amended water agar. The second group of seedlings are plated onto P5ARP, a selective media for *Pythium* spp. After the first group of seedlings have been allowed to incubate, growth isolations are made from the water agar and transferred onto antibiotic amended PDA. The seedlings are also removed and transferred onto TB-CEN, a selective medium for *Thielaviopsis basicola*. As for the second group of seedlings, they remain on the Pythium selective media until fungal growth sporulates. After all plates reach their incubation periods, they are examined under a compound scope for morphological structure identification to the genera level (Figure 1).

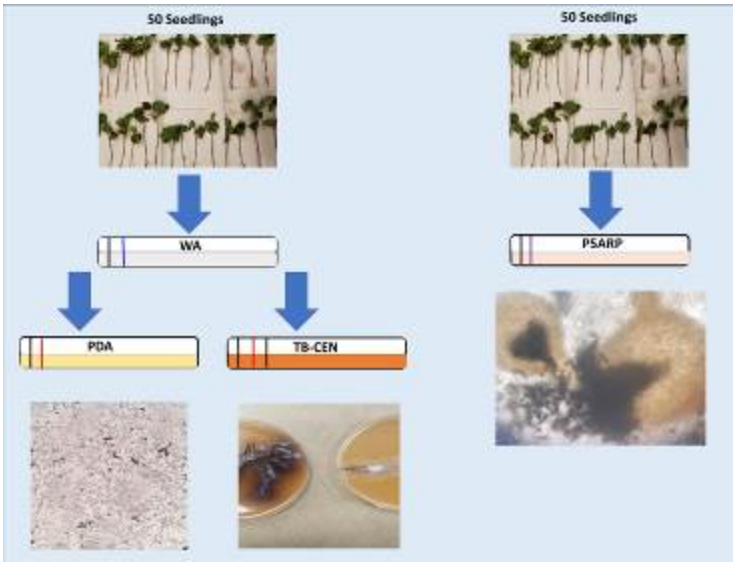

Figure 1. NCST seedling assessment plating process.

Soil Baiting

In addition to the 100 seedlings received for each location, cooperators are also responsible for sending in 10 soil cores from their non-treated checks. 500 grams of this soil is used for soil baiting for *Rhizoctonia solani*. In order to bait the pathogen, 4" pots are filled to the headspace with soil, which is saturated from the bottom and allowed to drain overnight. After the soil has been drained, 9 sterile flat toothpicks are inserted into the soil in a 3x3 square pattern. The toothpicks remain in the soil for approximately 48 hours in hopes that the fungus will adhere itself to the wood of the toothpicks. After 48 hours, the toothpicks are removed from the soil, and placed onto Terry Spurlock selective media. Each plate is gridded and contains 3 toothpicks each. Cells that exhibiting growth are recorded and then isolations are made from the growth and transferred onto antibiotic amended PDA. After 3 days the PDA plates are examined under a compound scope, for further confirmation of the pathogen (Figure 2).

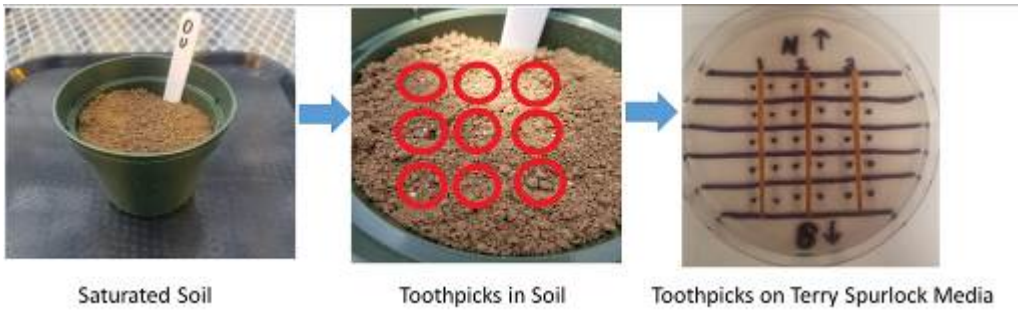

Figure 2. *Rhizoctona solani* toothpick baiting process.

Soil Dilution Plating

Finally, 25g wet or 30g of dry soil are used for soil dilution plating for each location. The soil is distributed into 500 ml screw top flasks, and 250ml of diluted water agar is added to the soil. The flask is placed on a wrist action shaker for about 20 minutes to allow the soil and water agar to incorporate. The soil dilution is distributed among 2 sets of plating for each location. The first set of plating is for *Thielaviopsis basicola*. For each location, 10 plates are used. 10mL are added to each plate, and TB-CEN is added to fill each plate using the pour plate method. After 1-2 weeks each plate is analyzed for growth and the number of colony forming units for each plate are recorded (Figure 3). The second set of plating is for *Pythium* spp. 6 plates are used for each location and 10mL of the original soil dilution are added onto premade P5ARP plates, and distributed across the plates using the spread plate method. After 48 hours, the surface of the plates are rinsed to remove any debris, and the number of colony forming units is recorded for each plate.

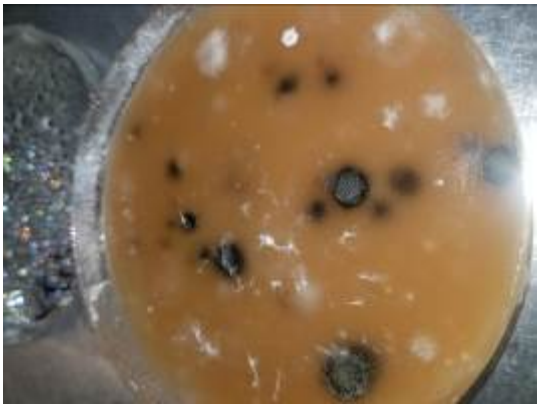

Figure 3. Colony forming units on TB-CEN.

Additional Measures

In addition to node counts, disease severity ratings, plating onto selective media, soil baiting, and soil dilution plating, there are several other measures taken to ensure that the data we are collecting is reliable. Weather data is collected to keep track of optimal growth conditions, remaining soil is used for parasitic nematode evaluation, and seed germination rates and stand count data are taken to analyze seed treatment efficacy.

Results and Discussion

Although the methods utilized in the current NCST protocol are adequate, the research team at the West Tennessee Research and Education Center will be removing some methods to increase efficacy and adding molecular, selective media, and/or enzyme-linked immunosorbent assay (ELISA) components to make the data more reliable. For example, adding a selective media for *Fusarium* spp., and discontinuing the use of TB-CEN media and soil dilution platings for *Thielaviopsis basicola,* and *Pythium* spp. analysis.

We will be testing a molecular and/or ELISA component to our protocol. Although there are several species of *Fusarium* and *Pythium* that are known to be pathogenic to cotton, we will only be seeking primers/ probes that are specific to genera for these pathogens. However, for *Thielaviopsis basicola* and *Rhizoctonia solani*, we will be seeking primers/probes that are specific to these species.

We will also be adding a new selective media for *Fusarium* species. There are many types of growth media that are known to be selective for *Fusarium* spp. But, we will be using Nash and Snyder medium (NS) and Czapek-Dox agar (MCz). Both of types of media are shown to produce higher isolation frequencies of *Fusarium* species that are known to be pathogenic to cotton.

As far as discontinuing some of the methods/techniques we currently use, we will no longer be using a selective media for *Thielaviopsis basicola*. This is due to the fact that the morphological structures of this pathogen are easy to identify and hard to confuse with other pathogens. Instead of using a selective media for the pathogen, we will be analyzing the roots directly under compound scopes. We will also not continue with the soil dilution plating for *Thielaviopsis basicola* and *Pythium* spp. The use of selective media has helped us identify these pathogens, and we do not see the need to pursue further confirmation through soil assays.

Summary

Moving forward with this trial, it is imperative that the purposed changes begin to be utilized. The use of node counts, disease severity ratings, seed germination testing, and selective media plating will be carried over into the new protocol. However, with the introduction of a molecular and/or ELISA component, a *Fusarium* spp. selective media, and the discontinuation of seedling plating onto TB-CEN and soil dilution plating will aid in continuing to provide data on pathogen populations associated with cotton seedling disease, and evaluate seed treatment efficacy in relation to these pathogens in a reliable and efficient fashion.

References

Andrews, S. and J. I. Pitt (1986). "Selective medium for isolation of Fusarium species and dematiaceous hyphomycetes from cereals." Selective medium for isolation of Fusarium species and dematiaceous hyphomycetes from cereals(6): 1235-1238.

Howell, C. R. (2002). "Cotton Seedling Preemergence Damping-Off Incited by Rhizopus oryzae and Pythium spp. and Its Biological Control with Trichoderma spp." Phytopathology 92(2): 177-180.

Johnson, L. F. and J. H. Doyle (1986). "Relationships of seedling disease of cotton to characteristics of loessial soils in Tennessee." Relationships of seedling disease of cotton to characteristics of loessial soils in Tennessee(3): 286-290.

Jones, W. M., et al. (2016). "Resistance to Rhizoctonia solani and Pythium ultimum in upland cotton (Gossypium hirsutum L.)." Crop Science 56(4): 1784-1791.

Roncadori, R. W. (1974). "Effect of Pythium irregulare on Cotton Growth and Yield, and Joint Action with Other Soil-Borne Pathogens." Phytopathology 64(10): 1303.

Zhang, J., et al. (1996). "Frequency of isolation and the pathogenicity of Fusarium species associated with roots of healthy cotton seedlings." Mycol. Res. 100: 747-752.