2012 Cotton Seedling Diseases: Importance, Occurance and Chemical Control

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The importance of seedling diseases on cotton, factors that influence seedling disease severity, and the efficacy of fungicides and treatment strategies for protection of cotton emergence and stands were examined in controlled environmental and field studies. Controlled environmental studies using soils that differed in pathogen populations, cropping history, and soil texture demonstrated that environment had a greater impact on the importance of seedling disease pathogens than pathogen population or field history. By examining stand difference between fungicide treated seed and black seed for over 50 sites from in-furrow fungicide studies, it was shown that temperature was the most important environmental factor in determining seedling disease severity, with lower soil temperatures at planting increasing seedling disease severity. Seed treatment fungicides consistently improved stands using data from the National Cottonseed Treatment Program and the efficacy of fungicides improved over time compared to a historical standard. In-furrow and custom-seed treatment studies from Arkansas, Louisiana, and Georgia were used to examine the value of these products to improve stand establishment. At minimal soil temperatures below approximately 18 ºC, a producer can expect an in-furrow fungicide application to improved stand approximately 80% of the time. Environment at or shortly after planting appears to be the major factor affecting cotton seedling disease severity, and predicting environmental conditions at planting can be used to improve the effective use of fungicide products.

Keywords: *seedling disease, fungicides, environment*

Cotton seedling diseases are a major factor affecting cotton production worldwide (DeVay, 2001; Hillocks, 1992; Melero-Vara and **Jimenaz-Diaz, 1990**. In the United States, loss estimates for seedling diseases accounted for 27% of the total estimated losses in lint production from 1991-2000 for diseases, including nematodes, even with the almost universal use of fungicide seed treatments (DeVay, 2001). The primary pathogens of the cotton seedling disease complex are *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris)*, *Thielaviopsis basicola* (Berkeley & Broome) Ferraris, *Pythium* spp., and *Fusarium* spp. Symptoms associated with seedling diseases include seed rot, preemergence damping-off, and postemergence damping-off. In addition to stand losses, seedling diseases may cause hypocotyl lesions and root rots that reduce plant vigor and delay early-season crop growth. This delay in early-season growth may result in additional management problems, such as timing of herbicide, insecticide, or fertilizer applications. In severe disease situations, replanting may be required. Although symptoms may be associated with specific pathogens, few diagnostic symptoms are available to make an accurate diagnosis of the causal agent or agents.

In addition to the prevalence of the seedling disease pathogens, soil environment plays an important role in disease. Soil temperatures and soil water during the first few weeks after planting are important in stand establishment because of effects on both the host and the pathogens (Johnson et al., 1969; Minton et al., 1982; Riley et al., 1969). Research in Louisiana has shown that poor stands and increased seedling disease are often associated

with early planting dates (Colyer et al., 1991). Early planting is often associated with lower soil temperatures and increased rainfall. Seedling diseases were negatively correlated with increasing soil temperature and positively correlated with increasing soil water by Johnson et al. (1969).

Cottonseed in the United States is universally treated with fungicides prior to sale, which is indicative of the severity of seedling disease problems and the efficacy of these fungicides. The grower can add additional fungicides either on the seed before planting (planter-box, hopper-box, or custom seed treatments) or in the planting furrow (in-furrow treatments). These practices should give greater protection to emerging plants and have been reported to be effective in controlling seedling diseases (Chambers, 1995; **Colyer and Vernon, 2005**; Minton and Garber, 1983, Minton, et al., 1982).

This talk will examine the importance of seedling diseases on cotton, factors that influence seedling disease severity, and the efficacy of fungicides and treatment strategies for protection of cotton emergence and stands.

MATERIALS AND METHODS

Controlled environmental studies. Eight soils were used to assess the role of pathogen populations and soil conduciveness under controlled environmental conditions. The eight field sites were selected because of their cropping history and range of soil textures (Table 1). For experiments, soil was collected at each site from the top 15 cm. The samples were refrigerated for four weeks or less. Two environments were used to assess disease among sites. The first environment (cool-wet) had a 12 hour photoperiod at a cyclic linear temperature regime between 16 and 21ºC with a matric potential between saturation and –10 Joules/kilogram (J/kg). The second environment (warm-dry) had a 12-hour photoperiod at a cyclic linear temperature regime between 20 and 26ºC with a matric potential between saturation and –30 J/kg. Soils were sieved through a mesh sieve and thoroughly mixed by hand and packed to a bulk density of 1.3 g/cm^3. Seven seed of the cotton cultivar Deltapine 451 B/RR were planted in each pot. Five replicates (pots) of each soil were used per controlled environment. Pots were weighed daily and watered gravimetrically back to saturation when pots dried below the desired matric potential range. Soil samples were assayed for populations of *Rhizoctonia* species by using the multiple-pellet soil method (Henis et. al., 1978), and *Rhizoctonia* populations were quantified on a modified Ko and Hora medium (Ko and Hora, 1971). Soil populations of *Pythium* spp. and *T. basicola* were detected by diluting 25 g of soil in 0.2% water agar to a total volume of 250 ml and placing on a wrist action shaker for 20 minutes. *Pythium* spp. were quantified by the spread-plate method on the selective medium P_5ARP (Jeffers and Martin, 1986) and *T. basicola* populations were quantified using the pour-plate method with the selective medium TB-CEN (Specht and Griffin, 1985), which was modified by adding Penicillin G (60 mg/L).

Daily measurements taken for each pot included plant emergence and damping-off. Three weeks after planting the plants were uprooted. Percent postemergence damping-off was calculated by subtracting stand at 21 days from stand at 7 days, dividing by the number of seed planted and multiplying by 100. For disease assessment and isolation, seedlings were then rinsed for 20 minutes in running tap water and rated for disease symptoms. The hypocotyl disease severity index was 1=no symptoms, 2=few pinpoint lesions or diffuse discolored areas, 3=distinct necrotic lesion, 4=girdling lesion, and 5=seedling dead. The root disease index was 1=no symptoms, 2=1-10% of the root system discolored, 3=11-25% of the root system discolored, 4=26-50% of the root system discolored, and 5>50% of the root system discolored. Seedlings were surface disinfested by immersion for 1.5 min in

0.5% NaClO, blotted dry in a paper towel, and plated on water agar (1.3%) amended with 10 mg and 250 mg of the antibiotics rifampicin and ampicillin, respectively, and 0.5 Φl of the miticide Danitol (Valent Chemical Co.) per liter. Resulting colonies were transferred to PDA and identified to genus. Seedlings were subsequently transferred to the *Thielaviopsis* selective medium TB-CEN to determine isolation frequency for *T. basicola.*

The experiment was conducted three times (runs) and data from all three experiments were combined where appropriate and were analyzed by the GLM procedure using SAS (SAS institute, INC., Cary, NC). Pearson product-moment correlation was used to determine correlation coefficients among soil, plant pathogen, and disease variables.

National cottonseed treatment studies. Cooperators in the National Cottonseed Treatment Program conduct field experiments annually across the U.S. Cotton Belt for the Cotton Disease Council. Data used in these analyses were from 1993-2004 and represent 214 trials. Each location utilized a randomized complete block experimental design, with the number of replications ranging from 4 to 10. Stand counts used in the analyses were generally taken at about 30 days after planting. A soil sample and seedling sample from plots containing nontreated seed were taken at the time of the stand count. Soil and seedlings were placed in insulated packages with refrigerated cool packs and mailed overnight to the University of Arkansas for processing. Disease indices and pathogen isolation were as described above. Each site was analyzed separately by the GLM procedure using SAS.

At each site, rainfall was measured by either a dry gauge on site or taken from recordings made at the nearest National Oceanic and Atmospheric Administration site (NOAA). Soil temperatures and soil water at sites were measured by burying a temperature sensor (Onset Computer Corp., Pocasset, MA) or Watermark (Spectrum Technologies, Inc., Plainfield, IL), respectively, 10 cm deep at planting, or in a few studies from an experiment station weather station.

In-furrow studies. Data from 118 in-furrow and custom-seed treatment studies from Arkansas, Louisiana, and Georgia were conducted at experiment station sites and in producers' fields. Seed was treated with a fungicide combination used by seed companies prior to planting. Commercial custom-seed and in-furrow treatments were registered products and used at labeled rates. Custom-seed treatment fungicides were compared to in-furrow fungicides and seed treatments in 38 tests. In 54 tests, black (nontreated) seed was included as an additional treatment to estimate seedling disease pressure. Production practices at each site followed extension production guidelines for that state.

The studies were performed between 1996 and 2004, with the number of sites per year ranging from 7 sites in 2004 to 16 sites in 1998. Replications ranged from three to eight and most sites were randomized complete block designs (91 sites). Row length counted for stand for most studies (98 sites) was 30.5 m, range 9.1 m to 60.0 m. A skip index (Chambers, 1986) was also taken for 79 sites and adjusted to 30.5 m. The skip index is a combination of the total number of skips in the planting row and the length of the skips. A skip is defined as a distance of 0.3 to 0.45 m of row without a plant, with the value increasing for each additional 0.15 m of skip length. Stand counts used in the analyses were taken between two and three weeks after planting. For experiment station sites, the middle two rows of the plots were harvested and recorded as seed cotton weight. For producers' fields the entire plot was harvested and weighed or recorded by yield monitoring equipment on the picker. Seed cotton yields were converted to lint yields by multiplying by 0.35.

Disease indices and pathogen isolation were as described above. Environmental data was collected by the same methods as for the National Cottonseed Treatment Program.

Each experiment was analyzed separately for each of the response variables recorded in that experiment based on its experimental design. For each response, *P*-values for the contrast of seed treatment verses the mean of all in-furrow fungicide treatments and the seed treatment verses the mean of all custom seed treatments were calculated and saved. Mean stand, skip, and yield were compared for the in-furrow products using analysis of variance based on a randomized complete block design with experiments as the blocking variable and product and form (liquid, granule) as the factors. The factors had an incomplete factorial treatment structure since some fungicides were present in both forms. Finally, mean responses for all in-furrow and custom-seed treatments were calculated for each experiment and compared using a randomized complete block design with experiment as the blocking variable and product type as the factor. RESULTS

Role of environment on disease.

The influence of environment on seedling disease severity was examined by comparing the difference in plant stands between fungicide-treated seed and black (nontreated) seed (Fig. 1). The mean difference was 15.6%, indicating the importance of seedling disease and fungicides in stand establishment. Differences in stand ranged from 3.2% to 38.1%. As temperature increased, seedling disease severity decreased. Increased rainfall had little impact on seedling disease severity.

The importance of pathogen populations or field history versus environment on seedling diseases was examined using eight soils in controlled environmental studies. *Pythium* populations differed among soils, range 54 to 533 CFU/g soil. For *R. solani*, pathogen populations differed significantly among soils, range 2.1 to 32.5 CFU/g. *T. basicola* populations ranged from 1.0 to 75.5 CFU/g soil. There were no significant differences for plant stand 21 days after planting among soils placed in a uniform environment. Plant stand ranged from 4.4 (63%) to 5.0 (71%) plants per pot for the cool-wet environment. Plant stand ranged from 4.4 (63%) to 5.2 (74%) plants per pot for the warm-dry environment. For the cool-wet environment damping-off for the soils averaged 11.2% and did not differ significantly among the soils, P=0.30. Damping-off for the soils ranged from 3.8% for soil A3 to 20.0% for soil C3 (data not shown). For the warm-dry environment damping-off for the soils averaged 10.1% and did not differ significantly among the soils, P=0.26. Damping-off for the soils ranged from 2.9% for soil C2 to 22% for soil A3 (data not shown).

Pathogen isolation data showed that there were no significant differences among soils for *Pythium* spp. or *R. solani. Pythium* spp. isolation ranged from 38.0% to 53.0% for environment 1 and 10.9% to 20.2% for environment 2. Isolation of *Pythium* spp. was favored by the cool-wet environment, which had a cyclic linear temperature regime from 16 to 21ºC with a matric potential between saturation and –10 J/kg, compared to the warm-dry environment. *R. solani* isolation ranged from 21.3% to 30.2% for environment 1 and 38.4% to 54.9% for environment 2. *R. solani* was favored by the warm-dry environment, which had a cyclic linear temperature regime from 20 to 26ºC with a matric potential between saturation and –30 J/kg compared to the cool-wet environment.

Pathogen isolation data showed that there were significant differences among soils for *T. basicola* isolation. *T. basicola* isolation ranged from 16.9% to 93.8% for environment 1 and

17.0% to 94.0% for environment 2. Soil A2 consistently had the highest isolation of *T. basicola* and soils A4 and K consistently has the lowest isolations among soils. Populations of *T. basicola* were positively correlated with isolation, r=0.772 (*P*=0.0005).

Efficacy of seed treatments. Of the 214 trials from 1993 through 2004 in the National Cottonseed Treatment Program, 120 trials **(56%)** had one or more fungicide combinations providing a significant increase in stand compared to nontreated seed (*P*=0.05**)**. These trials indicated the importan**ce** of seedling diseases and seed **treatment** responses **across** all regions, with fungicides improving stand in 8 of 10 trials in California in the western United States, 17 of 24 studies in Louisiana in the midsouth, and 7 of 12 studies in Georgia in the Southeast. Seed treatment chemistry has improved when compared to a historic standard, PCNB-carboxin (Vitavax) and metalaxyl **(**Allegiance or Apron). Products having a triazole fungicide had a stand of 61.1% and azoxystrobin 60.8%. Both groups of fungicides provided greater stands than combinations having PCNB (59.3%). As part of these trials, fungicides with specific activity were included to look at pathogen pressure. Of the 120 trials where a response was found, metalaxyl alone gave a stand increase in 40 trials (33%), indicating the importance of *Pythium* spp. in stand establishment at these sites. PCNB gave a positive response in 44 of these 120 trials or 37% of tests, indicating the importance of *R. solani* in these studies.

Value of custom-seed and in-furrow treatments. To assess the likelihood of in-furrow fungicide products providing additional protection of plant stands over seed treatment fungicides, the probability of an in-furrow response was plotted against environment, soil temperature and rainfall the first three days after planting. As minimal soil temperature decreased to below approximately 18°C, the probability of a stand increase from the use of in-furrow fungicides increased. At soil temperatures below approximately 18°C and no rainfall, the probability of a stand response was 0.2 or less, providing a stand response from the use of an in-furrow product of 4 out of 5 times or greater. As soil temperature increased, the likelihood of in-furrow products improving stands decreased. Probability values ranges from 0.0002 to 0.99. The mean probability was 0.36 (n=111).

The probability of an improvement in stand uniformity, skip index, from the use of in-furrow fungicides as influenced by environment was similar as the probability of response for stand. The probability of an in-furrow fungicide response generally increased with decreasing soil temperature and with no or high rainfall amount after planting.

Probability values ranged from 0.00004 to 0.95 for the graph. The mean probability was 0.40 (n=76).

Due to the low number of comparisons (n=35), the custom-seed treatment response figures over environment were not generated, but custom-seed and in-furrow application methods were compared. There was a reduction in the number of skips in the planting row from the use of in-furrow products compared to custom seed applications, 15.7 and 17.6, respectively, *P*=0.02 (n=31). Stand for in-furrow products and custom-seed treatments did not differ, 72.0 and 71.0, respectively, *P*=0.33 (n=35).

In-furrow products and product formulation, liquid versus granular applications, were compared and showed no consistent response of product formulation for plant stand. Chemistries for in-furrow products tested performed similarly. DISCUSSION

The cotton seedling disease complex consists of four main groups of pathogens, *Pythium* spp., *Fusarium* spp., *R. solani*, and *T. basicola*. All of these pathogens are ubiquitous in

fields with cotton production, with the exception of *T. basicola*. Fields in these studies all had a population of resident pathogens that support the suggestion that seedling disease pathogens are found in every field (Bird, 1973; Johnson et al., 1969). In a controlled environment, plant stand was not significantly different among the soils from different locations, indicating field history plays a minor role in seedling disease severity. Considerable disease was present in the studies based on symptoms. However, since seedling diseases on cotton are a complex, disease data is often difficult to interpret in terms of the importance of individual pathogens. Thus, isolation data was used as an indication of the role of a pathogen in seedling disease severity. Pathogen isolation data showed that *Pythium* spp. and *R. solani* had no significant differences among soils*. Pythium* spp. were favored by the cool-wet environment and *R.solani* was favored by the warm-dry environment. This indicates that the conditions of the environment had a greater influence on cotton seedling disease severity caused by these pathogens than soil populations. Huisman (1988) found that seedling disease associated with *Pythium* spp. were related to high soil water and low soil temperatures. Likewise, Arndt (1943) found that plants inoculated with *P. ultimum* at temperatures of 18°C and 20°C and 60% soil moisture had 100% plant death. Hunter et al. (1960) reported isolates of *R. solani* have different temperature optimums, but the most virulent isolates were most aggressive from 24 to 32ºC, with considerable disease still occurring at 20ºC. Pathogen isolation data showed that *T. basicola* had significant differences among soils. Since *T. basicola* is not ubiquitous in all soils, the differences among soils in natural field population experiments reflected soil populations of the pathogen. Holtz et al. (1994) found disease severity to be positively correlated with inoculum of *T. basicola.* Nehl et al. (2004) found that variation in populations of *T. basicola* accounted for 85% of the incidence of black root rot. Since the percent isolation of *Pythium* spp. and *R.solani*, the major pathogens in stand establishment, are not influenced significantly by field history or edaphic factors the major factor in stand establishment is the environment at or shortly after planting.

The importance of the environment shortly after planting is also indicated by the stand difference between the fungicide-treated seed and nontreated seed in the in-furrow studies. Using stand difference as an indicator of seedling disease, seedling disease severity decreased as temperature increased. While increased rainfall at or shortly after planting had little to no impact on seedling disease severity. This indicates that temperature seems to be the major factor affecting seedling disease severity. Garber et al. (1979) found similar results and concluded that for some locations, seed and seedling environment was poor enough to affect stands even though pathogen populations were not as high as other locations with less effect on stand. Colyer et al. (1991) in Louisiana showed poor stands and seedling diseases were often associated with early planting dates. Early planting is often associated with lower soil temperatures and increased rainfall.

The annual National Cottonseed Treatment Program conducted by the Cotton Disease Council in the United States has been valuable in establishing the importance of seedling diseases, specific seedling pathogens, and fungicides to cotton production in the United States. Fungicides seed treatments provided significant stand improvement in over 50% of trials indicating that seedling diseases are the most important factor in stand establishment. By the use of selective fungicides, these trials also have provided confirmation that both *R. solani* and *Pythium* spp. are important in causing stand losses across locations. Finally, fungicide chemistries for controlling seedling diseases continue to improve.

In-furrow fungicide studies have shown efficacy was influenced by environmental factors shortly after planting. At minimal soil temperatures below approximately 18ºC, producers have an 80% chance of an in-furrow application improving stand, or 4 out of 5 times.

Product formulation of in-furrow fungicides showed no consistent response. Insufficient data was available to look at probability of a response from custom-seed treatments; however, stand and skip indices suggest that these products are comparable or slightly less effective than in-furrow products.

These results suggest that the environment at or shortly after planting is the major factor affecting cotton seedling disease severity. The usage of seed treatment and in-furrow fungicide products can effectively control these diseases. Since the environment determines when seedling diseases will be a problem, the application of in-furrow fungicides should be based on minimal soil temperature at planting and on weather forecast.

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Table 1. Soil texture and field history for sites in the study.

Soil	Location	Soil texture	Percent (%) sand	Field history
A1	Ashley County, AR	silt loam	24	Cotton monoculture
A2	Ashley County, AR	silt loam	34	Cotton monoculture
A3	Ashley County, AR	sandy loam	56	Cotton monoculture
A4	Ashley County, AR	sandy loam	76	Cotton monoculture
C1	Clarkedale, AR	silt loam	34	Cotton monoculture
C2	Clarkedale, AR	silt loam	30	Cotton monoculture rotated to corn past three years
C3	Clarkedale, AR	silt loam	22	Cotton/hairy vetch sequence since 1972
K	Keiser, AR	sandy clay	48	Cotton monoculture

Figure 1. The influence of environment at planting on seedling disease severity using the difference between percent stand for the fungicide-treated seed and black (nontreated) seed.