Chapter 8

THE SEEDLING DISEASE COMPLEX ON COTTON

Craig S. Rothrock[1] and Michelle Schulz Buchanan[2]
[1]Department of Plant Pathology, University of Arkansas, Fayetteville, AR
[2] University of Arkansas System Division of Agriculture Cooperative Extension Service, Van Buren, AR

INTRODUCTION

Cotton seedling diseases are a major factor affecting cotton (*Gossypium hirsutum* L.) stand establishment and production worldwide (DeVay, 2001; Hillocks, 1992; Melero-Vara and Jimenaz-Diaz, 1990; Ogle et al, 1993). Cotton disease loss estimates for the United States over a ten-year period from 2003-2012 averaged 2.1% and loss estimates for seedling diseases accounting for 20% of the total estimated disease losses in lint production over these years, even with the use of fungicide seed treatments (Blasingame and Patel, 2013). Seedling diseases of cotton affect the germination, emergence, and early-season growth of cotton. In the field, the most obvious symptoms of cotton seedling diseases are the absence of plants, skips, in the planting row resulting from rotted seed or preemergence damping-off, the death of the seedling prior to emergence. In addition, seedlings may die within the first one to four weeks after planting from seedling diseases, postemergence damping-off. Poor stand uniformity has been shown to negatively affect yield. In the Rio Grande Valley of Texas, in single-drilled cotton, skips reducing plant populations by 25% lowered yields 13% and skips reducing plant populations by 40% lowered yields 26% (Heilman et al., 1976). Seedling diseases also may delay early-season crop growth and result in additional management problems. In severe disease situations replanting may be required.

A number of soilborne or seed-borne pathogens can affect cotton seedlings. These pathogens can act individually or in association with other pathogens as a part of the cotton seedling disease complex (Johnson, et al., 1969; Minton, et al., 1982; Rothrock et al., 2012). There have been more than 40 fungi isolated from diseased cotton seedlings, but only a few are considered primary pathogens (Hillocks, 1992). *Pythium* spp., *Rhizoctonia solani* Kühn, teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk, *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick) and *Fusarium* spp. are considered to be the primary pathogens of cotton seedlings (Colyer et al., 1991; Davis, 1975; DeVay, 2001; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Melero-Vara and Jimenaz-Diaz, 1990; Roy and Bourland, 1972; Rude, 1984). These pathogens cause a range of symptoms on roots and hypocotyls (DeVay, 2001; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Roy and Bourland, 1972). All of these groups of pathogens are ubiquitous in cotton soils, except *T. basicola*. Limited research has examined the importance of soil pathogen populations on disease development. DeVay et al. (1982) observed that survival of cotton seedlings from nontreated seed was negatively correlated with populations of *P. ultimum* in field soils. However, Davis et al. (1997) in California did not find an association between *R. solani* or *Pythium* soil populations and stands. In Tennessee, Johnson and Doyle (1986) also found no relationship between *Pythium* soil populations and isolation frequency or disease on cotton seedlings. The multi-year data from that National Cottonseed Treatment Trials found a low but significant negative correlation between *Pythium* soil populations and stand, but not other pathogens examined (Rothrock et al., 2012). However, soil population explained only a small portion of the variability in stands observed across trials. This information suggests soil pathogen populations are not the primary factor in determining seedling disease severity on cotton.

The importance of the environment and seedling diseases on cotton stand establishment have been examined as part of National Cottonseed Treatment Trials, a standardized fungicide seed treatment trial conducted by the Cotton Disease Council with cooperators across the cotton belt in the United States (Rothrock et al., 2012). The average minimal daily soil temperature and total rainfall the first three days after planting had a dramatic effect on mean stand across treatments, sites, and years for the 146 trials examined (Figure 1). Stand decreased as the average minimal soil temperature decreased from 24 to 12°C. As temperature decreased, increasing rainfall after planting had an increasing impact on limiting stands. The importance of seedling diseases on the stand reductions observed is evident in these same studies by examining the difference in percent stand between seed treatments having the combination of a triazole and either metalaxyl or mefenoxam fungicides and seed not receiving a fungicide treatment (Figure 2). Average difference in percent stand was 13.2 across all environments and stand difference increased dramatically as temperature after planting decreased and at lower temperatures as rainfall increased after planting. For these trials seedling diseases accounted for most of the stand differences observed across planting environments.

SEEDLING PATHOGENS

Pythium species

Seedling diseases caused by *Pythium* spp. are widespread in cotton-growing areas. The genus *Pythium* is in the Oomycetes which are now in the kingdom Stramenopila and are no longer considered fungi and include saprophytic and facultative parasitic species. Many species of *Pythium* contribute to the cotton seedling disease complex (DeVay et al., 1982; Howell, 2001; Johnson et al., 1978; Johnson and Chambers, 1973). The most common symptoms associated with *Pythium* spp. are seed rot and preemergence damping-off (Arndt, 1943; Howell, 2001; Howell, 2002; Spencer and Cooper, 1967). Younger seedlings (6 days old) were more susceptible to *Pythium* spp. than older seedlings (12 days old) (Arndt, 1943; Spencer and Cooper, 1967). *Pythium* spp. also cause root rotting and hypocotyl lesions

(Arndt, 1943; Howell, 2001). *Pythium* spp. can cause severe stand loss in cotton (DeVay et al., 1982; Fulton and Bollenbacher, 1959; Howell, 2001; Johnson et al., 1978; Ogle et al., 1993). Johnson et al. (1978) found that *Pythium* spp. were the most frequently isolated pathogen from diseased cotton seedlings in Tennessee. A significant negative correlation was obtained between percentage of seedlings from which *Pythium* spp. were isolated and percent emergence (Johnson and Doyle, 1986). The use of the fungicide seed treatment Metalaxyl, with selective activity against *Pythium* spp., in the National Cottonseed Treatment Trials examined the importance of *Pythium* spp. in the seedling disease complex (Rothrock et al., 2012). Of the 119 trials that had a fungicide response, metalaxyl increased stand significantly in 40 trials demonstrating the widespread importance of this group of pathogens in stand establishment.

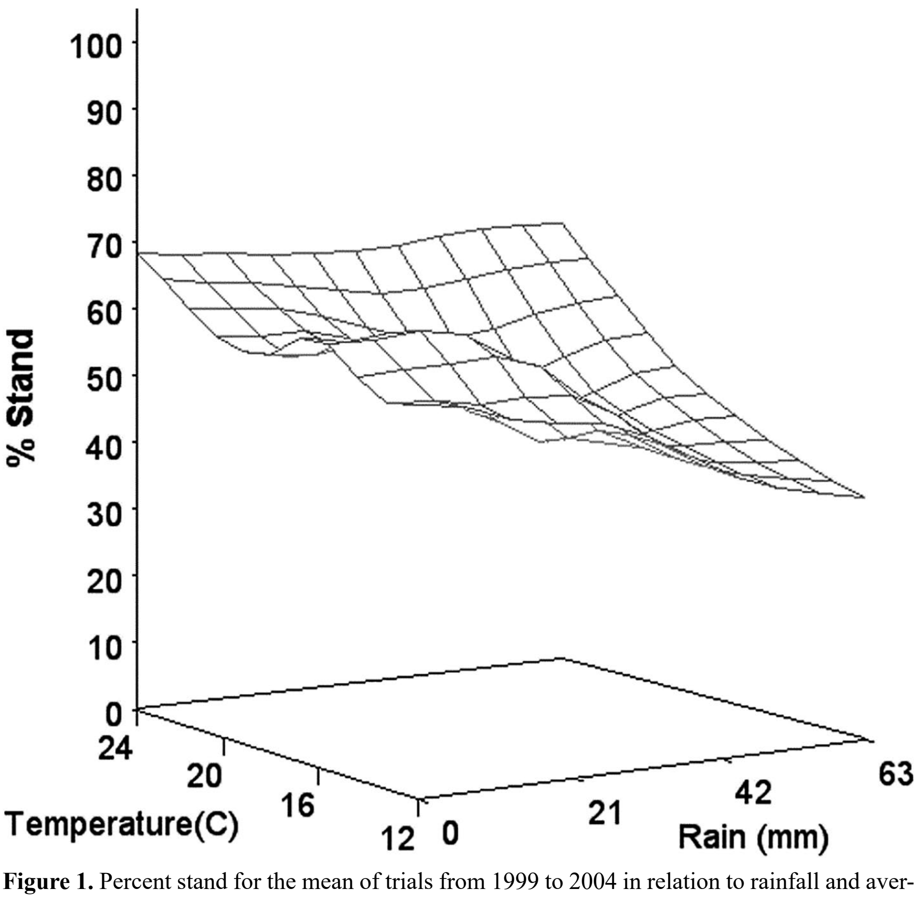

Figure 1. Percent stand for the mean of trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

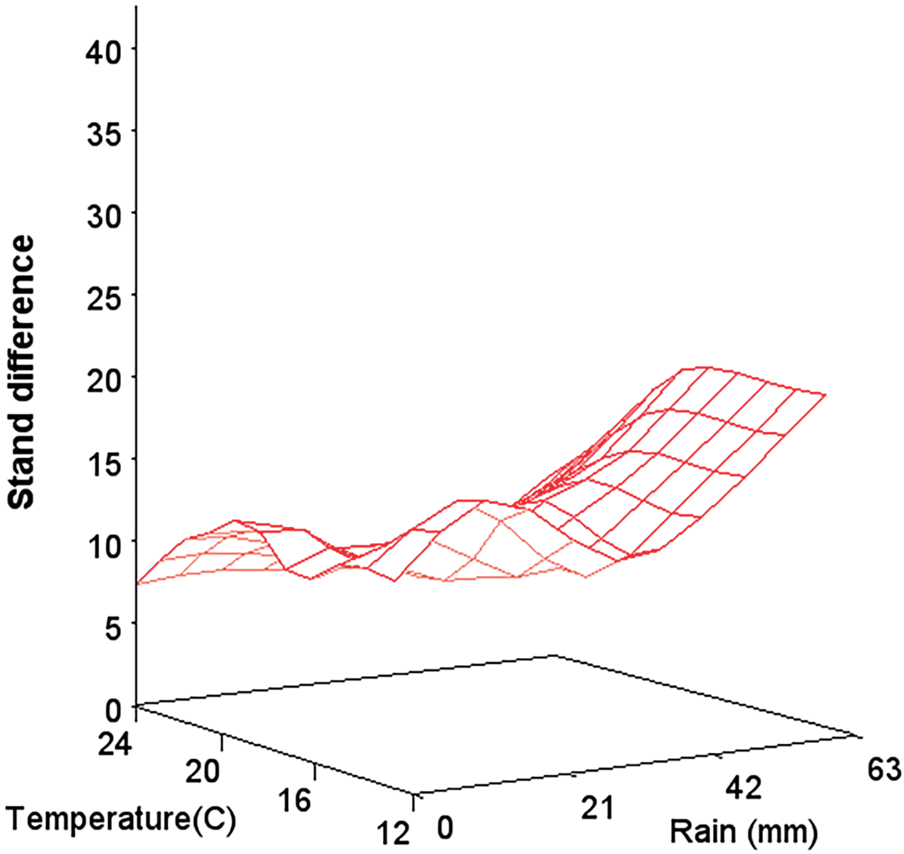

Figure 2. Difference in mean stand percentages between seed treated a triazole and either metalaxyl or mefenoxam and seed with no fungicide treatment for trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

The primary survival structures for *Pythium* spp. are oospores in the soil or in plant residue. The saprophytic nature of the pathogen also allows colonization of organic matter in the soil. A study by Nelson and Craft (1989) showed root and seed exudates stimulated germination of almost 100% of oospores of *P. ultimum* within three hours of initial exposure.

Losses from *Pythium* spp. increase as soil temperature decrease from a minimal soil temperature of 24 to 12°C and with increasing rainfall the first 3 days after planting (Figure 3) (Rothrock et al., 2012). These results are supported by earlier research from Tennessee that found in 7 years of field studies, soil moisture and temperature at planting directly affected isolation frequency of *Pythium,* with isolation being negatively correlated with minimal soil temperature and positively correlated with soil moisture content (Johnson et al., 1969). These observations on the role of soil temperature and moisture on the severity of seedling disease caused by different *Pythium* spp. also are supported by controlled studies. Optimal temperature for root rot in cotton caused by *P. irregulare* was 15°C, over a range of temperatures from 15 to 31°C (Roncadori and McCarter, 1972). Arndt (1943) found *P. ultimum* caused the most damage at 18 and 21°C over a temperature range of 18 to 30°C. McCarter and Roncadori (1971) found that germinated seed exposed to temperatures of 10°C for 3 or 5 days, which were planted in soil infested with *Pythium* spp., had reduced emergence compared to germinated seed not exposed to low temperatures. *Pythium* species have a range of temperature preferences, with *P. ultimum* growing well at low to moderate temperatures and *P. aphanidermatum* having an optimal temperature of 37°C (Howell, 2002). Growth of *Pythium* spp. is increased at higher soil water contents (Griffin, 1963; Hillocks, 1992). In addition, soil texture has been reported to affect *Pythium* spp. In Tennessee, *Pythium* was a greater problem in clay soils than in sandy soils (Johnson and Doyle, 1986).

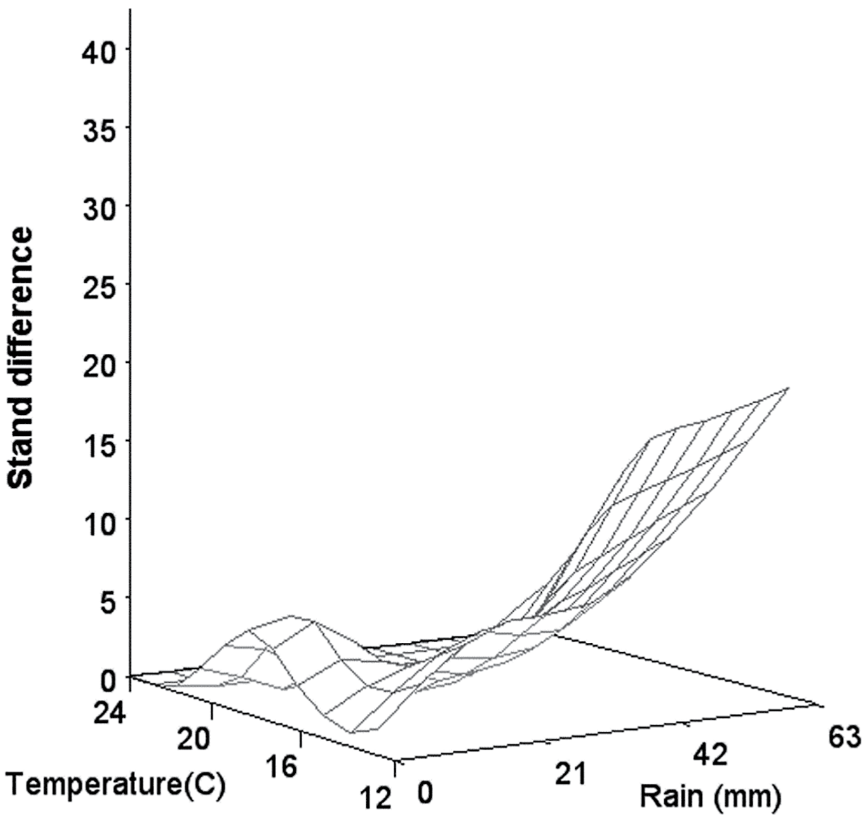

Figure 3. Difference in mean stand percentages between seed with a metalaxyl fungicide treatment and no fungicide treatment for trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

Rhizoctonia solani

R. solani is a common and important pathogen in the seedling disease complex of cotton. *R. solani* causes seed rot, preemergence damping-off, postemergence damping-off, as well as boll rots and leaf spots (Garber and Leach, 1971). Fulton and Bollenbacher (1959) and Ogle et al. (1993) reported preemergence death of cotton due to *R. solani*, However, the symptoms associated with seed rot and preemergence damping-off of *R. solani* are often hard to distinguish from symptoms of other seedling pathogens. The symptom most often associated wiith *R. solani* is postemergence damping-off (Garber and Leach, 1971; Rothrock, 1996; Rothrock, 2001; Rude, 1984) and include brown to reddish brown lesions found on the hypocotyl near or below the soil line. The lesions are usually sunken, referred to as "sore shin" or "wire-stem," and girdle the hypocotyl killing the seedling. Studies have shown that cotton becomes increasingly resistant to *R. solani* with age (Hunter et al., 1978; Neal, 1942). *R. solani* was reported to be the most important pathogen associated with diseased cotton seedlings in Mississippi (Davis, 1975; Ranney, 1962) and Oklahoma (Ray and McLaughlin (1942). The use of the fungicide treatment PCNB in the National Cottonseed Treatment Trials, which is selective for control of *R. solani*, increased plant stands over nontreated seed in 44 of 119 trials where a significant fungicide seed treatment response was found, demonstrating the widespread importance of *R. solani* in stand establishment.

R. solani reproduces and exists primarily as vegetative mycelium and/or sclerotia. It is a facultive parasite, which can grow saprophytically through the soil or on organic matter. *R. solani* is a very diverse species which is grouped below the species level into anastomosis groups (AG groups). This grouping is based on the ability of the hyphae of two isolates of the fungus to recognize and fuse with one another (anastomose). Isolates in an AG generally have similar host ranges and cause similar symptoms. Although the pathogen diversity on cotton has not been critically examined, Weinhold (1977) found that AG 4 was connected with soils historically used for cotton production. Rothrock et al. (1995) also found only AG 4 at two sites in Arkansas, both sites having a history of cotton production. Other AGs have been isolated from cotton and can cause symptoms on cotton under controlled conditions (Carling et al., 1994; Carling et al., 2002; Wrather et al., 2002).

Seedling disease in cotton caused by *R. solani* has been reported to be affected by temperature. Germinated seeds planted in soil infested with *R. solani* and exposed to a 10ºC temperature for 3-5 days, had reduced emergence compared to germinated seeds not exposed to the low temperatures (McCarter and Roncadori, 1971; Shao and Christianson, 1982). There was a negative correlation between temperature and exudates and a positive correlation between exudates and *R. solani* growth (Hayman, 1969). However, the results from the National Cottonseed Treatment Trials, which showed an average stand difference of 3.6 for PCNB treated seed compared to nontreated seed did not find much difference in fungicide response over minimal soil temperatures from 12 to 24°C and rainfall from none to 62 mm for the first 3 days after planting in 146 trials (Rothrock et al., 2012). Soil moisture has been shown to have little effect on the colonization of cotton plants by *R. solani* (Huisman, 1988; Johnson et al., 1969).

Thielaviopsis basicola

T. basicola, the causal agent of black root rot of cotton, has become an important seedling disease on cotton throughout the world (Allen, 2001; Johnson and Doyle, 1986; Mathre et al., 1966; Nehl et al., 2004; Roy and Bourland, 1982). *T. basicola* has a wide host range and is found in both agricultural and non-agricultural soils (Yarwood, 1974). Symptoms associated with *T. basicola* include blackening of the roots and the belowground portions of hypocotyls and stunting of cotton plants (Allen, 2001; Melero-Vara and Jimenez-Diaz, 1990; Rothrock, 1992). Generally, the damage is limited to the cortical layer and does not progress into the endodermis and the vascular cylinder (Allen, 2001; Mathre et al., 1966; Mauk and Hine, 1988; Walker et al., 1999). In severe cases, the development of the lateral roots is restricted or prevented (Allen, 2001). In Mississippi, *T. basicola* was detected in 18 of 36 locations and observed on 100% of cotton seedlings for four locations (Roy and Bourland, 1982). Surveys in Arkansas and Texas found *T. basicola* in over 70% of cotton fields (Rothrock, 1997; Wheeler et al., 2000). For surveys in Mississippi, incidence of *T. basicola* was negatively correlated with cotton stand and positively correlated with root and hypocotyl disease indices (Roy and Bourland, 1982). In microplot studies, soil infested with moderate levels of *T. basicola*, 100 chlamydospores/g soil, had lower yields in two of three years, reducing seed cotton yields 15 and 21% (Jaraba et al., 2013).

T. basicola is a hemibiotroph, functioning ecologically as an obligate parasite (Hood and Shew, 1997; Nan et al., 1992). The fungus produces two types of single-celled asexual spores, chlamydospores and endoconidia. The chlamydospores are thick-walled, dark, and produced in chains. The chlamydospores are the survival structures and primary inoculum of *T. basicola*. The fungus can survive as chlamydospores in the soil for more than 3 years and also may persist as endoconidia for up to 8 months (Allen, 2001). Under favorable conditions, the spores germinate and produce germ tubes in response to exudates from the plant roots. The pathogen directly infects the host plant and hyphae grow both intracellularly and intercellularly throughout the cortical cells (Allen, 2001; Mathre et al., 1966; Mauk and Hine, 1988).

Black root rot of cotton caused by *T. basicola* is more severe under cool environments after planting, less than 24°C (Allen, 2001; Blank et al, 1953; Maier, 1966; Rothrock, 1992). Black root rot is more severe in wet soils than in well-drained soils (King and Presley, 1942). At a temperature of 24ºC, soils with a higher matric potential (-10 J/kg), had 32% colonization of root tissue by *T. basicola*, compared to 12% at a lower matric potential (-30 J/kg) (Rothrock, 1992). Soil texture also has been suggested as important in the development of black root rot. Disease symptoms are often more severe in crops grown in clay soils as opposed to crops grown in sandy soils (Hillocks, 1992). Microplot studies comparing soil textures, found soil with 87% sand had less root colonization and reproduction and survival of the pathogen than soils with a range of sand contents from 48 to 74% (Jaraba et al., 2013).

Fusarium species

Fusarium spp. are commonly isolated from diseased seedlings and are frequently the most isolated genus of fungi from cotton seedlings (Colyer, 1988; Davis, 1975; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Johnson and Doyle, 1986; Melero-Vara and Jimenez-Diaz, 1990; Ray and McLaughlin, 1942; Roy and Bourland, 1982). A number *Fusarium* spp. are isolated from cotton seedlings (Abd-Elsalam et al., 2006; Colyer, 1988; Palmateer et al., 2004; Roy and Bourland, 1982). Roy and Bourland (1982) found a positive correlation between incidence of *Fusarium oxysporum* Schlechtend.: Fr. and disease severity on seedlings. However, Johnson and Doyle (1986) found a negative correlation between disease severity and isolation frequency of *Fusarium* spp. Pathogenicity for isolates of several species, most frequently *F. oxysporum* and *F. solani* (Mart.) Sacc. have been demonstrated (Abd-Elsalam et al., 2006, Colyer, 1988; Colyer, 2001; Melero-Vara and Jimenaz-Diaz, 1990; Roy and Bourland, 1982). Symptoms caused by *Fusarium* spp. include decayed seed, brown or black lesions on the hypocotyls and roots, and death of seedlings. Affected seedlings that emerge from the soil, often exhibit stunting, chlorosis, and reduced root systems. In the field, the effects of *Fusarium* spp. are similar to the other pathogens in the disease complex, in that they result in poor plant stands or skips (Colyer, 2001).

Species of *Fusarium* survive in the soil as chlamydospores or as hyphae. They can grow saprophytically in nature colonizing organic matter in the soil and can parasitize non-host plants, including weeds (Colyer, 2001). Species within the genus are distinguished based on the occurrence and appearance of macroconidia, microconidia, conidiophores, and chlamydospores.

SEEDLING PATHOGEN INTERACTIONS ON COTTON

In addition to seedling disease pathogens interacting to form a complex, these pathogens also may interact with other pathogens of cotton. Cotton seedling disease caused by *R. solani* was more severe in soils infested with the nematodes *Rotylenchulus reniformis*, *Hoplolairnus tylenchiformis*, *Meloidogyne incognita, M. arenaria, or M. hapla* (Brodie and Cooper, 1964). Susceptibility of cotton seedlings to *R. solani* also may be for a longer duration in plants previously infected by one of these nematodes. A synergistic interaction between *M. incognita* and *T. basicola* has been described in cotton (Walker et al., 1998). Microplots infested with both *T. basicola* and *M. incognita* showed an increase in seedling death and a decrease in plant growth and yield compared with microplots infested with either pathogen alone. Co-infection of cotton by both *T. basicola* and *M. incognita* appeared to increase the effective range of temperatures at which the pathogens caused damage (Walker et al., 1999). Histological studies showed the presence of *T. basicola* in the vascular root tissue in plants grown in soils where the fungus and *M. incognita* were present, suggesting the nematode allows *T. basicola* to colonize vascular tissues that are not normally colonized in the absence of the nematode (Walker et al. 1999). Early-season insect pressure from thrips also may increase seedling disease damage. Micinski et al. (1990) found that yields in Louisiana were increased across planting dates by the in-furrow application of Temik (aldicarb) plus Terraclor Super X (PCNB + etridiazole) compared to no in-furrow treatment suggesting early-season damage from thrips and seedling disease reduced yields. Yields across planting dates also were more stable by apply the in-forrow application compared to no in-furrow treatment suggesting early-season damage from thrips and seedling diseases were responsible for some of the yield variability over planting dates.

The interaction between *F. oxysporum* f.sp. *vasinfectum* and *M. incognita* is the most well-known nematode fungal interaction, being first reported by Atkinson in 1892. Atkinson (1982) observed that severity of Fusarium wilt of cotton increased in the presence of root-knot nematodes. *F. oxysporum* f. sp. *vasnifectum* also can interact with the reniform (*R. reniformis*) (Hillocks, 1992; Neal, 1954), sting (*Belonolaimus gracilis* and *B. longicaudatus)* (Holdeman and Graham, 1954; Minton and Minton, 1966), lance (*Hoplolaimus seinhorsti*) (Brodie and Cooper, 1964, Lewis and Smith, 1976; Rajaram, 1979), and lesion (*Pratylenchus brachyurus)* (Maggenti, 1981) nematodes. The interactions between the fungus and these nematodes increase the severity of Fusarium wilt of cotton, however, what role they play in seedling disease severity caused by *F. oxysporum* is unknown.

SEEDLING DISEASE CONTROL

Cotton seedling disease control relies on avoidance. Control practices used to limit seedling disease severity include planting high quality seed, planting in well drained soils on raised seedbeds, and delaying planting until soil temperatures and weather forecasts are favorable for rapid germination and emergence (Chambers, 1995; DeVay and Rothrock, 2001; Hillocks, 1992; Kerby et al., 1989; Minton and Garber, 1983, Minton, et al., 1982; Rude, 1984; Wheeler, et al., 1997). These practices should favor emerging plants and have been reported to be effective in controlling seedling disease (Chambers, 1995; Colyer and Vernon, 2005; Minton and Garber, 1983, Minton, et al., 1982). In addition, these practices decrease abiotic stresses, including soil crusting, soil anoxia, and chilling injury.

Early planting is a common practice for growers, in order to maximize the growing season, limit late-season insect pressure, and allow for favorable weather at harvest. Research in Louisiana has shown that poor stands and increased seedling diseases are often associated with early planting dates; with early April plantings resulting in low plant populations, late April and early May plantings resulting in intermediate plant populations, and mid-May plantings resulting in high plant populations (Colyer et al., 1991). Early planting is often associated with lower soil temperatures and increased rainfall, which are conditions favorable for cotton seedling diseases and delay plant emergence and development as shown earlier in the chapter for stand establishment (Figure 1). Soil temperatures, as well as soil moisture, affect both the host and the pathogens (Johnson et al., 1969; Minton et al., 1982; Riley et al., 1969). Johnson et al. (1969) found poor stands at minimal soil temperatures of 10°C or lower, with good stands at minimal soil temperatures of 19°C or higher. In Georgia, the efficacy of fungicide treatments was reduced when planting before March 29 or after May 2 (Guthrie, 1991).

Cottonseed in the United States is universally treated with combinations of fungicides prior to sale to protect the crop from a range of seedling disease pathogens, but also as a result of centralized seed production and sales due to the delinting process and the use of transgenic cultivars (Davis et al, 1997; Hillocks, 1992; Minton and Garber, 1983). It is common to use multiple fungicides to target the range of seedling disease pathogens that affect cotton. Davis et al. (1997) in California observed a stand improvement for the fungicides myclobutanil + metalaxyl or myclobutanil in 22 of 25 trials. Metalaxyl alone improved stands only in trials in 1995, but not the 18 trials in 1993 or 1994. In these studies, a fungicide response occurred over environments with mean soil temperatures that ranged from 19.7 to 22.2°C the first 5 days after planting, suggesting that seedling diseases control for adequate stands occurs over a range of planting environments. Kaufman et al. (1998) in Texas found seed treatments containing triazole fungicides improved cotton stands, reduced root necrosis, and increased yields. Wheeler et al. (2000) also demonstrated yield increases from seed treatment fungicides. The National Cottonseed Treatment Trials found improved stands from the use of fungicide seed treatment in 119 of 211 trials (Rothrock et al., 2012). New seed treatment chemistries combinations continue to be introduced and new products registered. For the control of *R. solani* and other pathogens, azoxystrobin and triazole fungicides, were shown to significantly improve stands when used in combination with mefenoxam or metalaxyl compared to the historical standard carboxin + PCNB + metalaxyl (Rothrock et al., 2012). Fichtner et al. (2005) observed that triadimenol with thiram and metalaxyl was more effective in reducing root necrosis and hypocotyl discoloration compared to seed treated with carboxin + PCNB + mefenoxam. Newer chemistries such as the triazoles are also increasing the spectrum of seedling diseases being controlled, with the triazoles providing some control of *T. basicola* (Kaufman et al., 1998; Toksoz et al., 2009). Additional control of seedling diseases may be achieved by the application additional fungicides either on the seed before planting (custom seed treatments) or in the planting furrow (in-furrow treatments).

SUMMARY

Seedling diseases are one the major factors limiting stands and stand uniformity in cotton early in the season. In addition to poor stands, vigor of surviving seedling may be poor delaying plant development. A number of pathogens cause seedling diseases including *Rhizoctonia solani Pythium* spp. *Thielaviopsis basicola* and *Fusarium* spp. These seedling pathogens are common in fields and thus the important factor in determine seedling disease severity in a given year and field are soil environment within the first few days after planting. Low soil temperatures and rainfall after planting increase seedling disease severity and reduce stands and stand uniformity. Control relies on avoiding disease pressure by creating a soil environment less favorable for disease. In addition, seed treatment fungicides have been used universally for years and the efficacy of fungicide seed treatments continue to improve in efficacy and the spectrum of pathogens they control.

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