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THE EFFECT OF SOILBORNE FUNGI ON YIELD STAGNATION

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Abstract

Yeld stagnation has been a concern of cotton producers in recent years. There are many reasons which may contribute to yield stagnation including the loss of breeding time caused by transgenic cotton varieties and the diminished root health because cotton is susceptible to soilborne fungi like *Rhizoctonia solani*, *Pythium* spp., and *Thielaviopsis basicola*. The interaction between *T. basicola* and the root-knot nematode is a common phenomena in sandy soils of the High Plains of Texas. This interaction causes a substantial reduction in lateral root length and health in approximately 30 % of the irrigated fields (600,000 acres) in this region. In years when spring rains are plentiful, tap roots may fail to develop properly. In some cases, this is due to *R. solani* or *Pythium* spp. rotting the tap root off, so that the plant is totally dependent on the lateral roots for anchoring, water, and nutrient uptake. Cotton plants transformed with a chitinase (ch5) or β-1,3 glucanase (bg2) gene were subjected to severe stress by these fungi. While these genes provided a slight improvement in plant stand compared with the nontransformed parent, the transformed plants appeared to be much more robust and increased yields by as much as 74 % over the nontransformed parent (754 versus 434 lbs of lint/a). Improved root health by transgenic or traditional breeding efforts may allow cotton varieties to reach their yield potentials.

Introduction

Yield stagnation has been a concern of cotton producers in recent years. The conversion of popular varieties in the early 1990's to transgenic versions is probably the most important reason that yields have not increased substantially in the last decade. Commercial breeders however are again expending considerable effort to increase the quantity and quality of cotton lint. With more money being spent on seed because of the added cost of herbicide or insect resistant genes, this has also increased the budget that cotton breeders have to work with. Breeders may be successful in developing varieties with better yield potential, but the ability of cotton to reach that yield potential is still being compromised by diseases. Very few commercial breeding programs are as determined to develop better tolerance to cotton diseases as they are to yield potential. This will likely result in few situations where varieties actually produce their potential.

In the High Plains of Texas, the disease complex caused by the fungus *Thielaviopsis basicola* and the nematode *Meloidogyne incognita* (root-knot nematode) affects approximately 30 % of the irrigated acreage (600,000 acres). While this fungus can severely stunt cotton seedlings and delay maturity in years when an extended cool spring occurs, it also causes a substantial reduction in lateral root length and health every year to cotton if the root-knot nematode is also present. In years when soil temperatures are warm > 75º F soon after planting, root necrosis caused by *T. basicola* is minimal and does not appear to affect seedling growth or maturity. However, as the population density of root-knot nematode increases, the damage to fine, lateral roots increases, so that in August there are almost no fine roots providing the plant with water. The fine lateral roots display a symptom of black tissue, and the roots are < ½" in length. This symptom can be found in every year when both organisms are present in a field, regardless of spring temperatures. A plant which is unable to obtain sufficient water will not reach its yield potential.

Management of root-knot nematode is either by crop rotation with peanut or with nematicides. Both of these methods can be effective at reducing the at-plant population density of the nematode. However, the nematode population does build up over the growing season. The symptom of tiny, black fine lateral roots was observed in cotton which had followed peanut. This would suggest that methods which reduce the at-plant nematode population density, but allow normal increase over the growing season, are not fully effective in stopping nematode damage. Root-knot nematode resistant varieties may be useful in improving root health under this disease complex. However, commercial varieties are not available with resistance to much of the cotton growing region. Acala NemX is an example of a variety with good resistance to the root-knot nematode. Effective management options for *T. basicola* are fewer than for root-knot nematode. Chemical control is nonexistent, though the use of certain seed treatments (triademenol or myclobutanil) can reduce root necrosis under low to moderate disease conditions. Crop rotation with grain crops for several years can also reduce the density of *T. basicola*, but in most cotton growing regions, a rotation of > 1

year is not practiced. There are no resistant cotton varieties to *T. basicola*, and the only resistance identified has been in diploid cotton species. Breeding for disease resistance to black root rot is of very low priority to the cotton industry. The fact that *T. basicola* resistant genes will be extremely difficult to transfer using traditional methods, and difficult to justify the cost using molecular methods, almost ensures that root health will continue to be compromised by this disease complex.

A deep tap root is important for cotton growth, particularly in water-limited environments, where deficit irrigation is practiced. Tap root size and depth can be affected by many factors including soil moisture, fertility, compaction, and soilborne fungi. Most commercial varieties are extremely susceptible as seedlings to fungi such as *Rhizoctonia solani* and *Pythium* spp. These fungi can rot seed when not sufficiently protected by fungicides. Once the plant emerges, then the systemic activity of fungicides may protect a limited area. However, much of the root system can be vulnerable to attack. As the plants mature they become relatively immune to damage by *R. solani* and *Pythium* spp. There is a period of time between emergence and when the root and stem tissue harden that the plants are vulnerable to fungal attack, and when fungicide protection is insufficient. In 2000, much of the High Plains of Texas experienced dry, warm weather during April and May (Figure 1A,1B). This encouraged producers to plant cotton early to conserve what soil moisture remained from rains or preplant irrigation. In June, approximately 2-3 wks after much of the irrigated cotton had been planted, a number of rainfall events occurred (Figure 1A). For some reason, possibly due to the extreme heat (Fig. 1B), cotton roots were not sufficiently mature in June to withstand *R. solani* and *Pythium* spp. In many fields, tap roots were destroyed within 4" of the soil surface. These fungi as well as others were consistently isolated from the rotted taproots. By three weeks after planting, there was no effective fungicide protection, and plants had no natural resistance to combat these fungi. The effects of these nonfunctional tap roots was seen throughout the growing season, and yields were low for fields with such limited root systems.

Most commercial varieties are very susceptible to *Pythium* and *R. solani*. While there are exceptions such as Acala Maxxa which has partial resistance to *Pythium* spp., the seed industry has chosen primarily to rely on chemical protection. Selection of breeding lines with resistance to *Pythium* spp. and *R. solani* is difficult. Field selections can only be made under conditions where significant disease occurs, i.e. under cool, wet conditions soon after planting. The distribution of these fungi in the soil is inconsistent, so that many "resistant" selections may only be escapes. Finally, the number of genes involved with resistance or partial resistance is not known, but may involve many genes, each with a small effect. These type of fungal pathogens, with wide host ranges and good saprophytic ability are often difficult to control with host resistance. Our understanding of host resistance is still in its infancy where pathogens like *R. solani* and *Pythium* spp. are concerned.

Another option to improve root health is by increasing the number or percentage of beneficial microbes in the soil. Some beneficial bacteria are able to lyse fungal mycelia. Bacteria or fungi which are used in biocontrol often contain chitinases, glucanases and other materials which are toxic or degrade plant fungal pathogens. It can be difficult to provide an environment where biocontrol fungi or bacteria have the advantage of colonizing a developing root over that of a pathogen. One method of using biocontrol without a biotic agent, is to incorporate one or more of the genes which are involved with a biocontrol agent into the cotton plant genome and express it constitutively. The plant may then be more resistant to fungi. This may be especially effective for root protection, which is so difficult to achieve with chemicals. An experiment was conducted on plants which had been transformed with either a chitinase (bean source) or β-1,3 glucanase (*Arabidopsis* source) gene to determine their ability to resist fungal attack.

Materials and Methods

The breeding line 95-T#20-1517 was supplied by Dr. John Gannaway (cotton breeder, Texas Agricultural Experiment Station, Lubbock, TX) to Dr. Ellen Peffley (Texas Tech University). Dr. Peffley and Zhixin Xiang successfully transformed the breeding line with a chitinase (ch5) or β-1,3 glucanase (bg2) gene. In 2001, there was sufficient seed to test one line transformed with chitinase and six lines transformed with β-1,3 glucanase, plus the nontransformed parent. These treatments were planted in a seedling disease trial located at Halfway, TX. The field was naturally infested with *R. solani* and *Pythium* sp., and seed was dusted (0.5 g) with *R. solani* (grown on oat seed) just before planting. The seed was treated with the general protectant fungicide Captan (1.5 oz a.i./100 lb seed). Each plot contained 100 seed planted over a 25' long and 3.33' wide area. There were four replications of each treatment arranged in a randomized complete block design. The test was planted on 1 May, and furrow irrigated with 4-5" of water on 3 May. Immediately after irrigation was finished it began to rain (Fig. 2A) and soil temperature dropped from a high of 76 ºF at one day after planting to 65 ºF or below from four through nine days after planting (Fig. 2B).

Plant stands were recorded weekly from 7 to 42 days after planting. At 28 days after planting, six root systems were removed and rated. Roots were rated for percentage of root necrosis (0 to 100 % scale), and for hypocotyl damage. The hypocotyl rating was from 0 to 3 with 0 = no damage, 1 = superficial lesion, 2 = sunken lesion, and 3 = sunken lesion which was killing the plant. Plots were hand harvested on 3 November, and a sample was ginned to obtain the percentage of lint and seed within each

harvested plot. Statistical analysis was performed by comparing transformed lines to the nontransformed parent using the Dunnett test ($P = 0.05$) for stand, root health, and yield.

Results and Discussion

The combination of rainfall and irrigation, plus cool temperatures within a few of days of planting resulted in high levels of seedling disease. Plant stands were unacceptable for all treatments (Table 1). Although five seed were planted per foot of row, < 2 plants emerged per foot of row, which would have indicated the need to replant in a commercial situation. There were more plants which survived for each of the transformed lines than for the nontransformed parent (Table 1), though differences were not significant. Root necrosis, primarily caused by *T. basicola,* was not affected by chitinase or β-1,3 glucanase genes (Table 1). There was less hypocotyl damage on the one line transformed with chitinase than for the nontransformed parent. All the lines with β-1,3 glucanase gene had an intermediate hypocotyl rating between the chitinase (more resistant) and nontransformed parent (more susceptible) (Table 1). At harvest, the transformed lines in general had a more robust appearance than the nontransformed parent. Yields were higher for all transformed lines than the nontransformed parent, though the differences were not significant (Table 1).

The transformed plants had only marginally better emergence than the nontransformed parent. The results were not sufficient to allow for the elimination of chemical protection. The decrease in hypocotyl damage by some transformed lines compared with the nontransformed parent was the only direct indication of improved disease resistance. The transformed lines in general had a much more robust appearance than the nontransformed parent. The added genes may have affected root health or plant vigor by reducing the colonization of pathogens, or possibly by stimulating other soil microbes. It may be beneficial to study these lines in situations where plant stand is not the main limiting factor to yield. These genes may provide cotton with better root health, though much more testing is necessary.

Table 1. The effect of cotton transformed with chitinase (ch5) or β-1,3 glucanase (bg2) on seedling disease caused by *Rhizoctonia solani* and *Pythium* spp.

Gene	Designation	Plants/foot[a] At 42 days After planting	% Root Necrosis	Hypocotyl Rating[b]	Lbs of Lint/a
none	95-T#20-1517	0.5	31	1.91 a	434
chitinase	CH27	1.2	33	1.04 b	754
glucanase	FA16	0.9	27	1.21 b	730
glucanase	FA20	1.2	33	1.45 ab	712
glucanase	FA24	0.9	34	1.25 ab	701
glucanase	FA26	1.1	35	1.33 ab	655
glucanase	FA49	1.0	25	1.37 ab	670
glucanase	FA50	0.8	27	1.54 ab	612

[a]Five seed were planted per foot of row.

[b]Hypocotyl rating was from 0 to 3 with 0 = no damage, 1 = superficial lesion, 2 = sunken lesion, and 3 = sunken lesion which was killing the plant. Significant differences were obtained between the nontransformed parent and the chitinase line, using Dunnett's test with $P = 0.05$.

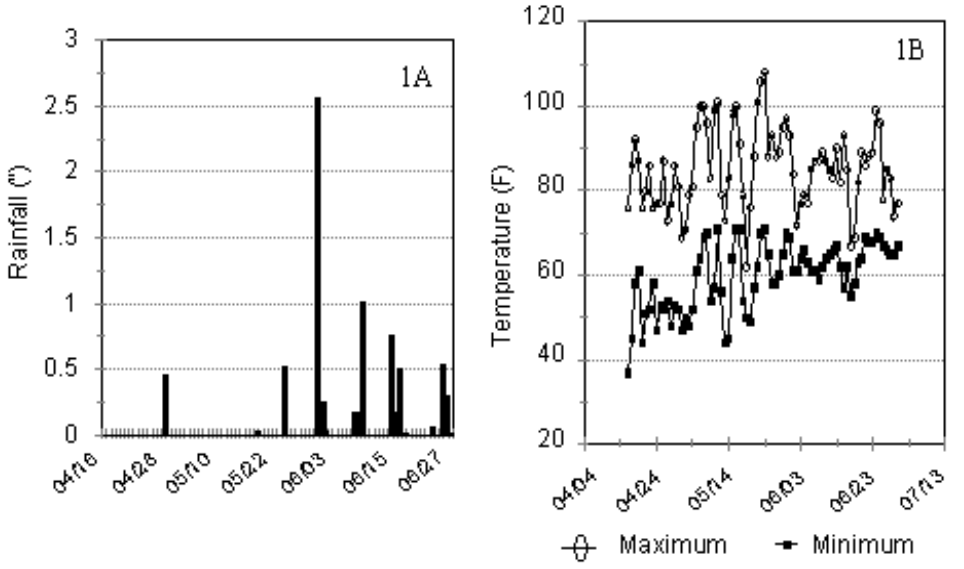

Figure 1. A) Rainfall which was recorded at the Lubbock Research and Extension Center during 16 April through 30 June, 2000. B) Maximum and minimum air temperatures recorded at the Lubbock Research and Extension Center during 16April through 30 June, 2000.

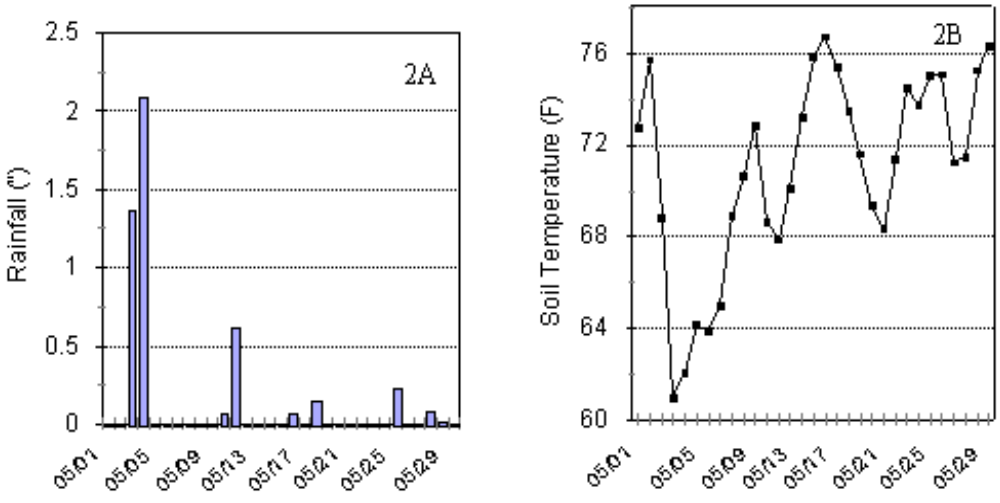

Figure 2. A) Rainfall which was recorded at the Halfway field station (Halfway, TX) during 1 May - 31 May, 2001. B) Soil temperature at a 4" depth which was recorded at the Halfway field station during 1May - 31 May, 2001.