

# ASSOCIATION OF *FUSARIUM* SP. WITH HARDLOCK OF COTTON IN THE SOUTHEASTERN US

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## Abstract

Hardlock of cotton is a widespread problem in the southeastern United States. Symptoms do not appear until the boll opens and the fiber does not fluff out, remaining instead compact and somewhat within the boll. Although the quality of the fiber is not severely affected, yields are reduced because conventional cotton pickers cannot harvest the fiber in the hardlocked bolls. The etiology of the disease is still undetermined, however it is associated with high nitrogen, high temperature and humidity, high plant density, insect damage, and seed rot. This study showed that fungi, especially *Fusarium* sp., isolated from the vascular elements of the peduncle, were also associated with the disease and that the amount of cotton actually harvested could be increased with applications of benomyl even when symptoms of Fusarium wilt of cotton were not present.

## Introduction

Hardlock of cotton seriously affects yield in many areas of the southeastern United States, especially in the panhandle of Florida. It is associated with high nitrogen, high plant density, high temperature and humidity, insect damage, and seed rot. Because hardlocked bolls are not harvested by conventional pickers, yields losses of up to 50% have occurred in the panhandle of Florida. A specific cause of the disease, if there is one, has not yet been identified. Extensive studies coordinated by Clemson University linked the occurrence of seed rot with hardlock and isolated a large number of bacteria after extensive sampling. However, no specific bacterial or fungal pathogen was identified (Jones, et al, 2000). The purpose of this research is to expand upon the effort to identify the casual agent of the disease and to attempt to develop effective control measures against the disease.

## Materials and Methods

The study was conducted on a Dothan sandy loam (fine loamy siliceous thermic Plinthic Kandudult), at the North Florida Research and Education Center in Quincy, Florida in 2001. Before planting the soil contained 26 ppm P, 118 ppm K, 133 ppm Ca and a pH of 5.6. The plot was previously in Bahia grass which was sprayed with 2,4 D at 1 pt/A plus Gramoxone at 1 pt/A plus Induce at 1 pt/A. Later Roundup was applied at 1.5 pt/A. The plots were ripped with a Ro-Till implement and planted. Two cotton varieties, Suregrow 501 BR or DP 458 B/RR, were planted with a Monosem planter at 4 seed per foot of row. The entire study was sprayed with the Roundup Ultra Max at 1.2 pt/A on 19 June. Cotton was broadcast sprayed with Cotoran at 1 pt/A + Bueno 6 at 1 pt/A on 27 June and sprayed with Staple at 1.6 oz/A + Induce at 1 qt/A on 11 July. On 17 July (42 days after planting at 1<sup>st</sup> square) cotton was side dressed with ammonium nitrate (34-0-0 of N-P-K). The entire study was broadcast sprayed with Pix at 1 pt/A + Agridex at 2 pts/A on 20 July and 1 week later direct sprayed with Direx 4 L at 1 qt/A + Induce at 1 qt/100 gal water. Cotton was broadcast sprayed with Pix at 1 pt/A on 2 August, Methyl Parathion at 1.5 qt/A + Decis at 2 oz/A on 22 August, and Orthene at 1 lb/A + Decis at 2 oz/A + Dimilin at 4 oz/A on 6 September. On 7 November cotton was defoliated with Dropp at 0.1 lb/A + Finish at 1.5 qt/A + Agridex at 2 qts/A. Cotton was picked with the International Spindle Picker on 7 December. The main effect was variety. The sub plots were no fungicide or 4 applications of benomyl (Benlate 1.5 lb/A). Sub-sub plots were treatment with nitrogen at 0, 60, and 180 lb/A. Each of the 6 row by 50 ft long plots were replicated 4 times.

At weekly intervals 20 bolls were picked from the first fruiting branch from each plot. A cross section was made and the bolls examined for seed rot. Healthy and diseased seed were selected for isolation in nutrient broth agar. The peduncle was examined for vascular discoloration by making a longitudinal cut. After a visual inspection the peduncles were plated on nutrient agar and 1/4 strength potato dextrose agar. Both seeds and peduncles were surface sterilized for 2 minutes with 10% sodium hypochlorite before plating. Representative bacteria were identified by fatty acid methyl ester analysis using the MIDI system (MIDI, Inc., Newark, DE).

Seven isolates of commonly encountered fungi (6 *Fusarium* sp and 1 *Pestalotia* sp were inoculated into the stems of DP 458 B/RR within 1 inch of the main branch 1 month before harvest, before the first boll on the branch had opened. Inoculation was accomplished by injecting a spores approximately 0.1 ml of spore suspension containing 50,000 spores per ml with a hypodermic. Autoclaved water was used injected as a control. At harvest each branch was removed and the amount of hardlock on each boll determined.

## Results and Discussion

There was no direct relationship between the frequency of microorganisms isolated and the appearance of diseased tissue in the seed and peduncle isolations during the season (Table 1). Overall, there were more bacteria isolated from the seeds and more fungi isolated from the peduncles. The most prevalent fungus was *Fusarium* sp., accounting for more than 80% of the fungi isolated. Other fungi isolated included *Cheatomium*, *Pestalotia*, *Cunninghamella*, *Nigrospora*, *Aspergillus* and *Phoma*. Bacteria isolated included *Agrobacterium radiobactor*, *A. Rubi*, *Cedecea davisae*, *C. Lapagei*, *C. Neteri*, *Escherichia coli*, *Enterobacter cancerogenus*, *E. Gergouiac*, *Klebsiella pneumoniae*, *K. Trevisanii*, *Kluyvera ascorbata*, *Pseudomonas aureofaciens*, *P. Putida*, *Salmonella typhimurium*, *S. Choleraesuis*, *S. Typhi*, *Serratia plymuthica*, and *Stenotrophomonas maltophilia*.

When commonly encountered fungi were inoculated into the stems of cotton plants, the incidence of hardlocked locks in bolls increased (Table 2). All of the fungal inoculated plants had an increase in hardlock as compared to the control, however vascular discoloration in the peduncle was rarely observed. The average number of hardlocked locks in the fungal inoculated branches ranged from 1.62 to 2.57, significantly more than the control with 0.74.

Yield of seed cotton in the test plots were significantly affected by the rate of nitrogen and the application of benomyl. (Table 2). Although the benomyl did not significantly increase yield in the lowers rates of nitrogen application, there was a significant increase in yield when benomyl was applied to plots that received 180 lb/A of nitrogen. Variety did not affect yield, so the yields were combined.

## References

Jones, M. A., J. D. Mueller, D. A. Kluepfel, M. J. Sullivan, J. T. Walker, Jr., M. E. Roof, J. McD. Stewart, and D. E. Linvill (Eds). 2000. Preliminary investigations on cotton seed rot in South Carolina. Clemson University Station Bulletin 675. 21 pp.

Table 1. Frequency of isolations from healthy and diseased seeds and peduncles.

		<u>Seeds</u>			
<u>Variety</u>	<u>Status</u>	<u>Bacteria</u>	<u>Bacteria and Fungi</u>	<u>Fusarium</u>	<u>Nothing</u>
DP 458 B/RR	Diseased	0.30	0.08	0.27	0.35
	Healthy	0.33	0.07	0.18	0.42
Suregrow 501BR	Diseased	0.37	0.06	0.25	0.32
	Healthy	0.32	0.01	0.22	0.45
		<u>Peduncle</u>			
<u>Variety</u>	<u>Status</u>	<u>Bacteria</u>	<u>Bacteria and Fungi</u>	<u>Fusarium</u>	<u>Nothing</u>
DP 458 B/RR	Diseased	0.05	0.04	0.51	0.40
	Healthy	0.10	0.11	0.51	0.28
Suregrow 501BR	Diseased	0.11	0.10	0.37	0.42
	Healthy	0.09	0.10	0.44	0.38

Table 2. Effect of benomyl applications and nitrogen on yield of cotton.

<u>Cotton Lint Yield (lb/A)</u>		
<u>Nitrogen (lb/A)</u>	<u>No Fungicide Applied</u>	<u>Four applications of benomyl</u>
0	1314	1300
60	1307	1354
180	1056	1208