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ETIOLOGY, EPIDEMIOLOGY, AND CONTROL OF FUSARIUM HARDLOCK OF COTTON IN THE SOUTHEAST: THE POSSIBILITIES

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Abstract

The primary symptom of hardlock of cotton is during boll opening the fiber does not fluff out but instead stay in tight wedges, often not extending beyond the boll. Mechanical pickers cannot extract the cotton fiber and the lock itself is often knocked to the ground. Hardlock of cotton is a complex disease that apparently can be induced by several different phenomena. It is associated with stink bug damage, seed rot, and - the emphasis of this paper - the fungus *Fusarium verticillioides* (formally *F. moniliforme*). We propose that hardlock of cotton be considered a subset of the more general boll rots, with a major difference in that with hardlock the fiber does not fluff out when the boll opens, but the boll itself is not decayed as in traditional boll rots. At present it is impossible to predict if a boll will fluff out normally before opening. We feel that Fusarium hardlock, as a subset of the hardlock complex, can be controlled with appropriate fungicide applications.

Introduction

Hardlock of cotton (*Gossypium hirsutim* L.) occurs when seed cotton adheres in compact wedges within the individual partitions, or locules, of matured, opened cotton bolls. In hardlocked bolls the lint has failed to fluff out; the individual fibers are not loose and white, but interlaced in discolored, frequently grayish, locule-shaped crescents (Fig. 1.). Although the properties of the fiber may not be grossly affected, the consistency of the seed cotton mass is such that conventional spindle picking equipment is not able to extract the fiber from the locules. In conventional mechanical harvesting, hardlocked cotton often is knocked to the ground or strung out of the boll, giving the appearance of poor harvesting procedures. Hardlock can seriously reduce yields in many areas of the southeastern United States, and in some years (e.g. 2002 with a yield loss of over %50) has been devastating in the panhandle of Florida. The incidence of hardlock has been associated with use of high rates of nitrogen fertilizer, high plant densities, high temperature and humidity during the growing season, insect damage, and in South Carolina, with seed rot (Jones et al., 2000; Marois et al., 2002; Marois and Wright, 2003).

In this work the symptoms of hardlock are differentiated from the traditional boll rots. In boll rots the carpel wall turns brown or black and the boll may be shed. If it remains attached it may fail to open or open partially. Insects can impact boll rot by damaging the boll through feeding and/or by transmission of pathogens. *Fusarium* spp. are often associated with boll rot of cotton but are usually considered secondary invaders. Sparnicht and Roncadori (1972) reported that *F. oxysporum* and *F. roseum* initially infected the bracts of uninjured bolls and then invaded the capsule through the receptacle on 5-week-old bolls. They reported that basal boll rot in Georgia was predominantly caused by *Fusarium* spp.

The objectives of this study were to determine the cause and evaluate possible control strategies for hardlock of cotton.

Materials and Methods

Field Plots

All studies were conducted on a Dothan sandy loam (fine loamy siliceous thermic Plinthic Kandiudult) at the North Florida Research and Education Center in Quincy, Florida in 2001 and 2002. In 2001, cotton varieties 'Suregrow 501 BR' and 'Delta Pine and Land (DPL) 458 B/RR' were used; in 2002, DPL 555 B/RR was used. All varieties were genetically engineered to be resistant to glyphosate herbicide and to produce the *Bacillus thuringiensis* endotoxin. The crop was maintained according to the recommendations of the University of Florida unless otherwise noted. Orthene and methyl parathion were used when needed to control the southern green stinkbug and the brown stinkbug. Cotton was harvested with a spindle plot picker.

Organisms Associated with the Interior of Cotton Bolls

In 2001, 20 bolls were picked from the first fruiting branch from plots planted to cotton varieties DPL 458 B/RR and Suregrow 501BR at weekly intervals for 8 weeks. Bolls were cut transversely and examined for seed rot and other symptoms. Apparently healthy and diseased seed were selected for isolation and identification of microorganisms. The subtending peduncle was examined for vascular discoloration by making a longitudinal cut. After visual inspection, seeds and peduncles were surface sterilized by soaking for 2 minutes in 0.6% sodium hypochlorite solution and plated on nutrient agar or 1/4 strength potato dextrose agar. Isolated fungi were identified by microscopic examination of phenotypic features. Representative bacteria were identified by fatty acid methyl ester analysis using the MIDI system (MIDI, Inc., Newark, DE).

Inoculation of Stems and Flowers

In 2001 and 2002, the potential of *F. verticillioides* to incite disease was tested. In 2001 a hypodermic syringe was used to inject one of five different isolates of the fungus, originally obtained from cotton, into the stem of 'DPL 458 B/RR' 2.5 cm below the peduncle of 20 2- to 3-week-old bolls and were tagged with date of inoculation. Approximately 0.1 ml of spore suspension containing 5.0 X 10^4 spores/ml was injected into the stem. Sterilized water was used as the control. The experiment was repeated 8 times during the 8 weeks of bloom, each time on a different set of plants. At maturity, each lock (seed cotton in the interior of an individual locule) of the 20 bolls per treatment was rated for the incidence of hardlock. The percent hardlock in each boll was calculated by dividing the number of locks exhibiting hardlock by the total number of locks in the boll.

In 2002, 'DPL 555 B/RR' cotton flowers were inoculated with the same five isolates of *F. verticillioides* used in 2001. Approximately 0.3 ml of a spore suspension containing 5.0 X 10^4 spores/ml was misted into a flower on the day of opening. Twenty flowers were treated with each isolate. At maturity, the bolls were rated for locks exhibiting hardlock. Fungi were re-isolated from the diseased bolls. The experiment was repeated 8 times over 8 weeks during the season.

In the winter of 2002, a greenhouse inoculation experiment was conducted with 'DPL 555 B/RR' cotton grown in pots. Flowers, upon day of opening, were inoculated by misting 0.3 ml of a spore suspension containing 5.0 X 10^4 spores/ml. Control flowers on separate plants were treated with sterile water only. Fifteen plants were used for each treatment and all of the flowers on each plant were used. Severity of hardlock was determined as in the field. Fungi were re-isolated from the diseased bolls as previously described.

Effect of Fungicides on Fusarium Hardlock

In 2001, a split-plot field trial was initiated with the main effect of cultivars ('Suregrow 501 BR', a short season cultivar, and 'DPL 458 B/RR', a long season cultivar). Sub plots were no fungicide or 4 applications of benomyl at 1.2 kg a.i./ha at the 4^{th}, 5^{th}, 6^{th}, and 7^{th} week of bloom . Nitrogen applications were added as an effect. Sub-sub treatments were nitrogen at 0, 67, and 201 kg/ha. Each of the 6 rows by 15 m long plots was replicated 4 times. Yields were obtained by harvesting 2 adjacent 6 m long rows with a mechanical spindle picker.

A field experiment was conducted in 2002 to determine if protection of flowers with fungicides was as important as protection of the bolls during opening. A randomized complete block design was used. Thiophanate-methyl, which has the same mode of action as benomyl, was applied at 1.0 kg a.i./ha for each application. 'DPL 555 B/RR' was planted in 4 row by 15 m long plots. The four treatments were: 1) weekly application during 6 weeks of bloom, 2) three applications weekly after 75% boll opening, 3) weekly application during 6 weeks of bloom and three applications after boll opening, and 4) no fungicide application. Each treatment was replicated 4 times. Yields were obtained by harvesting 2 adjacent 6 m long rows with a mechanical spindle picker. Disease severity was determined by rating 25 plants in each plot. The total number of locks with hardlock was divided by the total number of locks in all the bolls on all 25 plants. The result was a percent hardlock index for each plot.

Impact of Weather on Fusarium Hardlock

Fifty individual flowers of 'DPL 555 B/RR' were tagged on the day of bloom (white flower) eight different times in 2002. The bolls resulting from those flowers were rated for hardlock. Temperature and relative humidity were recorded every 15 min, 24 hours a day, during the entire season with a Campbell Scientific CR10 micrologger utilizing a combination temperature and humidity sensor (model HMP45C, Campbell Scientific, Logan, Utah). Linear regression analysis of disease severity to temperature and relative humidity was conducted to help quantify the potential of weather conditions to affect severity of hardlock.

Results

Organisms Associated with the Interior of Cotton Bolls

There was no direct relationship between the frequency of microorganisms isolated and the appearance of visibly diseased tissue in the seed and peduncle during the season. Overall, more bacteria were isolated from the seeds and more fungi isolated from the peduncles. The most prevalent fungus was *Fusarium* spp., accounting for more than 80% of the fungi isolated. Further analysis showed that over 80 to 85% of the isolates of *Fusarium* were *F. verticillioides*.

Inoculation of Stems and Flowers

Five different isolates of *F. verticillioides* obtained from cotton bolls in 2001 were inoculated into the stems of cotton plants near the peduncle in 2001. The severity of hardlock increased significantly in inoculated plants (Table 1). Overall, the fungal inoculated bolls had an average of 52% hardlocked locks, while the water inoculated control had only 18%. Even in the inoculated bolls, vascular discoloration in the peduncle was rarely observed. The fungus was consistently re-isolated from inoculated bolls, seeds, fiber and peduncles.

One-hundred seeds were collected from both diseased and healthy bolls and germinated in the greenhouse. Twenty-five days after planting, 52% of the seeds from the healthy bolls germinated, whereas only 22% of the seeds germinated from diseased bolls.

In 2002, the same five isolates of *F. verticillioides* were applied to flowers by misting with a spore suspension on the day they opened. Twenty flowers were included in each treatment and the injections were made 8 times during the season. The severity of hardlock increased significantly with the inoculation of the fungus (Table 1). The average severity on the bolls from inoculated flowers was 80%, significantly greater than the 55% in the water inoculated flowers. There were no differences in hardlock associated with the isolates used.

In the greenhouse trials, 58% of the locks were hardlocked from flowers inoculated with *F.* verticillioides, significantly greater than 32% in the water treated flowers. The pathogen was re-isolated in over 75% of the seed and fibers and 15% in the controls.

Effect of Fungicides on Disease

Natural disease pressure was greater in 2002 than in 2001. In 2001, the severity of hardlock was not affected by the fungicide treatments and ranged from 10 to 20% in the plots. Yield of seed cotton in the test plots in 2001 were significantly affected by the rate of nitrogen and the application of benomyl (Table 2). Since cultivar did not affect yield, yield data were combined. There was a significant interaction of fungicide and nitrogen rate. Benomyl did not significantly increase yield at the lesser rates of nitrogen, but significantly increased yield (from 1184 to 1354 kg/ha) when applied to plots that received 200 kg/ha of nitrogen.

In 2002, natural disease pressure was very high and the application of thiophanate-methyl significantly reduced the severity of hardlock and increased yield (Table 3). Fungicide applications made during the 8 weeks of bloom were the most effective at controlling hardlock and resulted in a 87% yield increase over the unsprayed plots (1345 and 717 kg//ha, respectively) while applications made during boll opening increased yield over the control by approximately 30% (975 kg/ha). Fungicide applications made at both bloom and boll opening did not increase yield significantly over those achieved with only bloom application (1367 kg/ha), but did further decrease disease. Across treatments, the severity of hardlock was indirectly correlated with yield (yield = 5672 - % disease*60.8, r = 0.86, n = 15) (Fig. 2). Cotton seed weight (Table 3) was increased significantly with applications of fungicide during the bloom period.

Impact of Weather on Disease Development

Disease severity (percent locks with hardlock from tagged flowers) ranged from 40 to 95% from flowers flagged during the 8 weeks (Fig. 3). There was a significant correlation between disease severity and temperature and relative humidity from 0700 to 1900 hr on the day flowers opened. The regression model was:

Predicted Disease = -2.67862+temp*0.08444+rh*0.01404 during 0700 to 1900 on day of bloom, $p < 0.005$, $r = 0.935$, $n = 8$, where Predicted Disease = proportion of locks with hardlock, temp = temperature in ^{o}C, and rh = percent relative humidity.

Discussion

In this study, *F. verticillioides* was discovered to be a fungal pathogen infecting cotton flowers and developing further in the boll as it matures. The fungus was able to further infect the cotton seed, reducing seed weight and germination, and peduncles, sometimes leading to discoloration of the vascular system of the peduncles. Although the pathogen is seed-borne, it is not obvious that this is important in the epidemiology of the disease, as the pathogen is widespread in the environment and is often associated with decaying plant material (Bolkan et al., 1979). No information was found on the application of fungicides to flowers in an attempt to control hardlock of cotton, however Roncadori et al. (1975) proposed that fungicides applied to flowers may be important in the control of *Fusarium* induced boll rot.

The importance of hardlock in reducing yield was shown in 2002. Hardlock was very severe in the Florida panhandle in 2002, and the average yield was reduced from a five year average of 730 kg/ha to only 388 kg/ha, due almost entirely to hardlock. When the hardlock cotton that was knocked to the ground during harvest was collected, ginned and weighed, it was found that it accounted for most of the reduction in yield.

Flower thrips (*Franklinella* spp.) may also play a role in the development of hardlock. Thrips numbers were reduced by traditional insecticide programs, and the potential role of thrips in damaging cotton flowers or serving as vectors of pathogens has not been investigated. Farrar and Davis (1991) reported that controlling thrips with insecticides was an effective way to manage corn ear rot also caused by *F. verticillioides*. Recently Gitiatis et al. (2003) found that tobacco thrips (*Frankliniella fusca*) transmit the bacterium *Pantoea ananatis*, which causes center rot of onion. High numbers of thrips were observed in cotton flowers during these experiments (data not shown) and may have the potential of carrying pathogens into the flower.

The high correlation between hardlock severity and the atmospheric conditions on the day of bloom further indicates the importance of flower infections in the epidemiology of the disease. Many other pathosystems involve the infection of reproductive structures by *F. verticillioides* (Michailieds and Morgan, 1998; Munkvold et al., 1997; Rohrbach and Pfeiffer, 1976; Vanstaden et al., 1989). Endosepsis of fig (*Ficus carica* L.) is caused by *F. verticillioides* and is spread from pollinator figs to edible figs by a small wasp (*Blastophaga psenes* L.) (Michailieds and Morgan, 1998). Mango malformation symptoms are due to the production of cytokinins

by *F. verticillioides* after infection of flowers (Vanstaden et al., 1989). It may be possible to develop a disease forecast model that will help growers determine when fungicides need to be applied to manage the disease effectively.

Further studies are needed to determine the timing and rates of different fungicides for optimum management of the disease. Because in the southeastern United States cotton blooms and produces bolls that are capable of maturing for 6 to 10 weeks, there is a potential need to protect flowers from infection over a long period. Accordingly the best strategy may be application of systemic compounds or systemic resistance inducing compounds. It may be possible to focus on the earlier flowers or first 4 weeks of bloom, since these contribute the most to yield in some production systems (Jenkins, et al, 1990).

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Table 1. Effect of inoculation of *F. verticillioides* on hardlock disease of cotton.

Isolate	Percent Hardlock 2001[x]	Percent Hardlock 2002[y]
Fusarium verticillioides 1	63 a [z]	80 a [z]
Fusarium verticillioides 2	54 ab	81 a
Fusarium verticillioides 3	53 ab	82 a
Fusarium verticillioides 4	51 ab	81 a
Fusarium verticillioides 5	41 b	79 a
Control	18 c	55 b

[x] Bolls were inoculated by injecting the fungus into the stem just below the peduncle.
[y] Flowers were inoculated by spraying a spore suspension on them on first day of bloom.
[z] Numbers in a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 2. Effect of benomyl applications and nitrogen on yield of cotton in 2001.

Nitrogen (kg/ha)	Cotton Lint Yield (kg/ha)	
	No Fungicide	Fungicide
0	1472 a [z]	1457 a
67	1465 a	1517 a
201	1183 a	1354 b

[z] Numbers in the same horizontal row followed by the same letter are not significantly different according to orthogonal comparisons.

Table 3. Effect of thiophanate-methyl applications on disease, lint yield and seed weight of cotton in 2002.

Treatment	% Disease	Yield (kg/ha)	Seed Weight (g)
Control	62 a[z]	717 c[z]	3.62 c[z]
Boll Sprays	48 b	975 b	3.76 b
Bloom Sprays	45 b	1345 a	3.97 a
Boll & Bloom Sprays	35 c	1367 a	4.06 a

[z] Numbers in a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Figure 1. Cotton boll exhibiting symptoms of hardlock.

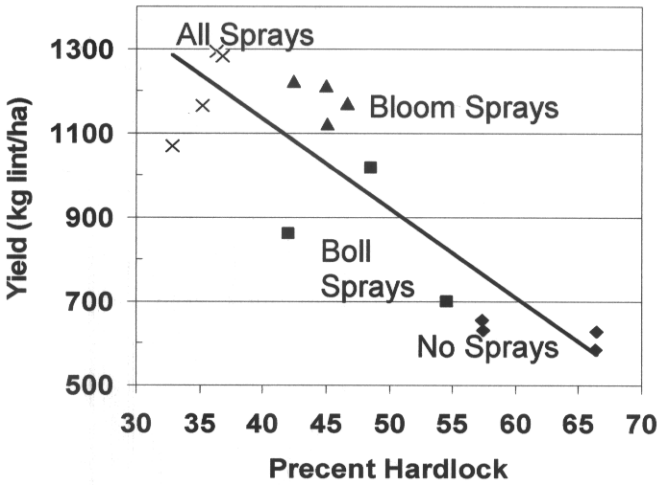

Figure 2. Relationship of disease and yield across fungicide treatments. (Yield = 1985 – Disease * 21.3, $p < 0.001$, $r = 0.86$, $n = 15$). Boll Sprays received three application of thiophanate-methyl weekly after bolls opened. Bloom Sprays received 6 weekly applications of thiophanate-methyl during the bloom period. All Sprays received both bloom and boll sprays.

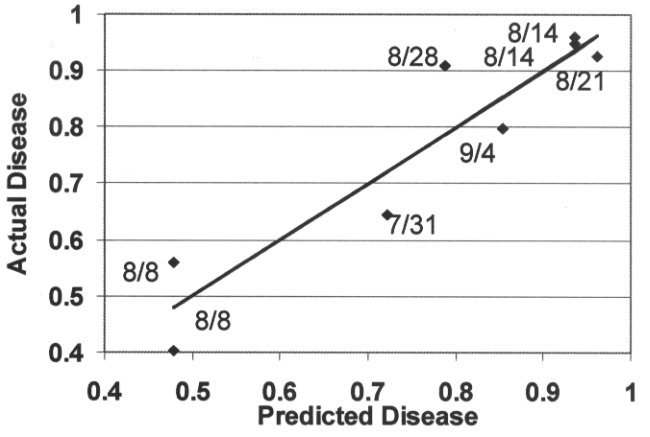

Figure 3. Relationship of disease and weather conditions on day of bloom. (Predicted Disease = -2.67862 + temp * 0.08444 + rh * 0.01404 during 0700to 1900 on day of bloom, $p < 0.005$, $r = 0.935$, $n = 8$). Inserts are date flower bloomed.