FUSARIUM HARDLOCK ON COTTON - ETIOLOGY AND CONTROL

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Abstract

Hardlock of cotton is a significant factor in the loss of harvestable yield in Florida. A project to further understand the etiology, epidemiology, and management of hardlock of cotton has been underway in Florida since 2002. It was determined that the fungus *Fusarium verticillioides* is a major contributor to hardlock in Florida, and this particular subset of the hardlock phenomenon was named Fusarium hardlock of cotton. Further research determined that the pathogen was able to infect the boll through the cotton flowers and that insect visitors to flowers contributed to the development of disease. Although *F. verticillioides* produces the mycotoxin fumonisin, fumonisin was not detected in a survey of diseased bolls, possibly due to the high levels of oils in cottonseed. This paper is a summary of our results thus far. Insecticide plus fungicide combinations applied during the bloom period of the cotton provided the best control and greatest yield increase.

Introduction

Hardlock of cotton (*Gossypium hirsutum*) occurs when seed cotton adheres in compact wedges within the individual partitions, or locules, of matured, opened cotton bolls. Often the fiber quality is not severely affected, but conventional spindle picking equipment can not harvest bolls with tight locules. Hardlock can seriously reduce yields in the coastal areas of the southeastern United States. The incidence of hardlock has been associated with high rates of nitrogen, high plant densities, high temperature and humidity during the growing season, insect damage, and seed rot (Jones, et al, 2000, Marois, et al, 2002, Marois and Wright, 2003, Wright, et al, 2004). *Fusarium verticillioides* (formerly *Fusarium moniliforme*) was frequently isolated from small lesions in bolls, from the peduncle, and from seed and fiber (Marois, et al, 2002).

The presence of *F. verticillioides* raises the possibility of cotton seed being infected with the mycotoxin fumonisin. Cotton seed is already regulated for aflatoxin, however other mycotoxins have not been identified as being a problem. The information on fumonisin in cotton seed is incomplete, however growers have faced litigation for *Fusarium* contaminated seed. In general, seed contaminated with *Fusarium* toxins are from severely delayed harvest (Cotty, 2001).

The optimum control strategy developed thus far for Fusarium hardlock is application of fungicide, or a combination of fungicide/insecticide, to cotton blooms (Marois, et al, 2005). Experience by researchers and growers indicate that there is a decrease in hardlock and increase in yield when applications are applied during bloom. If applications are repeated on a weekly or biweekly schedule, protecting more of the blooms from infection, yield continues to increase. Reduction of fungal leaf spots associated with the fungicide applications has also been correlated with increased yield.

Materials and Methods

Field Plots

Field 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. DPL 555 B/RR was used, 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. Cotton was harvested with a spindle plot picker.

Fumonisin Studies

F. verticillioides isolates from cotton bolls were grown on three different media to determine their potential to produce fumonisin. Acidified potato dextrose agar (aPDA) was used as the standard control whereas Acidified cotton flower agar (aCFA) and Acidified cotton flower agar (aCFA) were used in an attempt to simulate the nutrient conditions available to the fungus during infection.

Acidified potato dextrose agar (aPDA) was prepared according to standard methodology. Potato dextrose agar (Difco), agar, and water were combined and autoclaved. After the mixture cooled sufficiently, lactic acid was added to inhibit bacterial growth.
Acidified cotton seed agar (aCSA) is no longer available commercially, cotton seed meal (Greenfire Inc.; Chico, CA) was substituted. Cotton seed meal was ground to a fine consistency using a commercial-grade blender (Waring; Torrington, CT). The cotton seed meal was then added to water, and stirred vigorously for 10 minutes. The cotton seed meal was then filtered from the water using cheesecloth (Fisher Scientific; Pittsburgh, PA). Dextrose and agar (Difco) were then added. The media was autoclaved, and lactic acid was added to inhibit bacterial growth.

Acidified cotton flower agar (aCFA) was prepared based on the recipe for aCSA. White, first-day cotton flowers were collected on and immediately stored at -15ºC. They were then freeze dried for 72 hours at 1/2000 ATM of pressure and -80ºC. Afterward, they were finely ground using a commercial-grade blender (Waring; Torrington, CT). The ground cotton flowers were then added to water, and stirred vigorously for 10 minutes. Solid particles were filtered from the water using cheesecloth (Fisher Scientific; Pittsburgh, PA). Dextrose and agar (Difco) were then added. The media was autoclaved, and lactic acid was added to inhibit bacterial growth.

To determine the differences in resources available to the Fusarium between the media samples of freeze-dried cotton flowers and cotton seed meal were sent to Waters Agricultural Laboratories, Inc. (Camilla, GA).

Samples of fungus or plant tissue were also tested for the presence of fumonisin utilizing the ELISA system from Romer Labs (Union, Mo). The system is well-based microtitre plate ELISA kit using a direct competitive ELISA with a horseradish peroxidase conjugate as the competing, measurable entity. Its lower threshold is 0.2 ppm.

Results

Open bolls were collected from field plots and examined for the presence of fumonisin using ELISA. Bolls were rated for severity from 1 (low – only one locule with symptoms) to 4 (all locules with symptoms). The lower threshold of the assay was 0.2 ppm. No fumonisin was detected in the field samples, although when isolates of *F. verticillioides* originating from cotton bolls were grown on aPDA over 5 ppm of fumonisin was detected (Table 1).

Table 1. Presence of the mycotoxin fumonisin in cotton.

Sample Source	ppm of fumonisin[a]
Healthy bolls	not detected
Type 1 (low severity)	not detected
Type 2 (low-moderate severity)	not detected
Type 3 (moderate severity)	not detected
Type (high severity)	not detected
Fusarium verticillioides 1 from aPDA	5.61
Fusarium verticillioides 2 from aPDA	5.85

[a] minimum detection level is 0.2 ppm

Growth of *F. verticillioides* was not affected by the different media (Fig 1) even though the media were very different in their chemical composition (Table 2). However, the production of fumonisin was reduced

by ½ on the cotton based media as compared to the aPDA media (2.33 ppm for aCSA, 2.00 ppm for aCFA, and 4.00 ppm for aPDA.

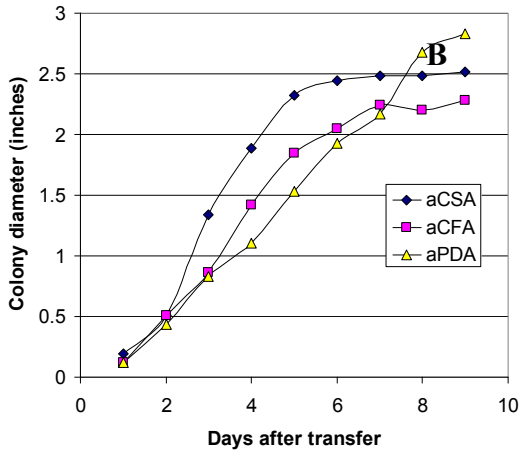

Figure 1. Colony growth of *F. verticillioides* was not significantly affected by media. aCSA is acidified Cotton Seed Agar; aCFA is acidified cotton flower agar, and aPDA is acidified Potato Dextrose Agar.

Table 2. Chemical analysis of the media used.

Media Analysis	PDA	CSA	CFA
Crude Protein	3.76%	15.10%	6.96%
Crude Fat	0.45%	1.35%	1.73%
Nitrogen	0.59%	2.36%	1.10%
Phosphorus	0.03%	0.38%	0.16%
Potassium	0.17%	0.70%	1.16%
Magnesium	0.10%	0.22%	0.10%
Calcium	0.22%	0.17%	0.17%
Sulfur	0.53%	0.37%	0.30%
Boron	73 ppm	56 ppm	62 ppm
Zinc	4 ppm	22 ppm	21 ppm
Manganese	2 ppm	9 ppm	5 ppm
Iron	43 ppm	97 ppm	17 ppm
Copper	1 ppm	7 ppm	19 ppm

Discussion

Since 2002, results from field and laboratory studies indicate the value of managing Fusarium hardlock of cotton. One concern was the possibility of the causal agent, *F. verticillioides*, to produce the mycotoxin fumonisin. Our findings indicate that this is a low risk, possibly due to the chemical make up of the cotton flowers and cotton seed.

Based on several years experience with growers, we are recommending that growers with sever hardlock problems in the southeastern U.S. try to manage the disease with 2-4 applications of fungicide or

fungicide/insecticide combination. As with any new practice, try this in a portion of the field or in strips across the field so that a comparison can be made with present practices. In our experience growers can expect an average increase of around 250 lbs/acre. From an economic perspective, the chemicals (thiophanate-methyl and orthene) plus application would be around $50/acre for 3 applications. With a return of 250 lb/acre, at $0.70/lb, the net return would be $125 per acre.

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