Influence of Fungi on Cotton Seed Deterioration Prior to Harvest

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University of Georgia College of Agriculture Experiment Stations Journal Series Paper No. 1064.

Supported in part by a grant from Cotton Incorporated, Raleigh, North Carolina.

Accepted for publication 1 June 1971.

ABSTRACT

Seed collected from commercially harvested, fluffed locks previously exposed to moist periods were deteriorated and showed a high degree of fungal infection. The frequency of isolation of fungi from samples taken at 13 different locations averaged 60.5% from whole seed and 32.0% from embryos. Seed germination averaged 45.5%, and the mean free fatty acid content was 6.3%. Poor seed germination was significantly correlated with high levels of embryo infection by the total fungal population, *Fusarium* spp., and *Diplodia gossypina*. High free fatty acid content was significantly correlated with frequent isolation of the total fungal population and *Fusarium* spp., both from the whole seed and the embryo. After 5 months' storage at 15 C and seed moisture content of 7-9%, the total fungal population and *Fusarium* spp. in the whole seed, but not in the embryo, were reduced significantly. Sufficient inoculum was still present to cause a seedling disease problem the following growing season. Phytopathology 61: 1326-1328.

Additional key words: Gossypium hirsutum.

Deterioration of cotton seed (*Gossypium hirsutum* L.), from boll opening to harvest, is a serious problem when the environment is unfavorable (13). Moist periods during boll dehiscence favor both boll rot development(6) and autocatalytic changes within the seed (10). Partially infected locks resulting from boll rot contain seed that are usually infested with fungi (5). Consequently, they may be of limited use for planting or milling purposes. These microorganisms can be designated as field fungi, based upon their ecological habit (3).

Improvements in cultural practices have brought about changes that may favor the activities of field fungi. The use of mechanical harvesters in recent years has dictated that most of the boll crop be opened and fluffed prior to picking. As a result, much of the seed cotton is exposed to the field environment for 1 month or more, affording an opportunity for fungal invasion. Little is known about fungal infection of seed in fluffed locks and their effect upon seed deterioration prior to harvest.

We conducted studies to determine (i) the fungal microflora present in the seed of mechanically harvested, fluffed locks; (ii) whether fungal invasion was correlated with seed deterioration as measured by germination and free fatty acid content (FFA) of the seed; and (iii) the capacity of the fungi to survive until the following growing season under conditions favorable for seed storage. A preliminary report on a portion of this work has been published (11).

MATERIALS AND METHODS.–From the last week in September through November 1969, commercially harvested, fluffed seed cotton was collected from 13 locations in the Georgia Coastal Plain and Piedmont. Samples were taken immediately after harvest, either in the field or at the gin, to minimize storage effects prior to ginning. At seven of the 13 locations, commercially ginned seed of the same lots were also collected. The seed cotton was air-dried and hand-ginned. Within 1 week after sampling, all seed were delinted in concentrated H_2SO_4 for about 3 min and washed in running water for 5 min. Seed moisture content was adjusted to 7-9% by air-drying. Moisture content was determined by oven-drying the seed at 100 C for 24 hr. The samples were stored in double polyethylene bags at 15 C.

Viability was determined by germinating 200 seed from each sample in sterile sand at alternating temperatures of 30 C for 8 hr and 20 C for 16 hr. Tests were terminated after 12 days. Initial germination was determined ca. 4 weeks after collection to reduce the effect of dormancy (12). Analysis of FFA in the seed oil was done by a commercial seed-testing laboratory.

Four hundred seed from each lot were assayed for microorganisms, of which 300 were processed as whole seed. Isolations were made from the remaining 100 seed by soaking them in tap water for 30 min, removing the seed coats, and plating the embryos. Both whole seed and embryos were surface-sterilized in a solution of 0.525% sodium hypochlorite and 5% ethanol for about 2 min, drained, and placed on 2% water agar in petri dishes. The samples were kept at room temperature and initially observed after 4 days. Isolates were transferred to potato-dextrose agar (PDA) for further identification.

A 12 x 12 correlation coefficient matrix was calculated to determine the relationships between seed lot characteristics and fungal invasion. Data on changes in seed germination and survival of fungi in storage were analyzed, using Duncan's multiple range test.

RESULTS.–Excess rainfall during field exposure, particularly during September (14), was associated with the poor quality of seed at most sampling locations. Germination ranged from 11.0 to 81.0%, and averaged 45.5% (Table 1). FFA content of the seed varied from 1.5 to 15.3%, and averaged 6.3%.

TABLE 1. Germination, free fatty acid content, and occurrence of fungi in hand-ginned cottonseed samples at harvest

Seed lot	Germination, %	Free fatty acids, %	Isolation of fungi, %							
			Whole seed				Embryo			
			Total fungi	*Fusarium*[a] spp.	*Diplodia gossypina*	*Glomerella gossypii*	Total fungi	*Fusarium* spp.	*Diplodia gossypina*	*Glomerella gossypii*
1	11.0	4.9	27.0	9.3	5.7	0.0	20.0	4.0	5.0	0.0
2	11.5	14.9	69.0	54.7	9.3	0.0	62.0	50.0	6.0	0.0
3	47.5	7.6	80.0	50.3	20.0	0.0	34.0	16.0	3.0	0.0
4	36.0	7.1	87.0	67.7	5.3	0.0	45.0	27.0	4.0	0.0
5	34.0	15.3	88.0	61.3	4.0	4.0	45.0	33.0	1.0	7.0
6	21.0	11.8	82.0	50.2	14.3	2.0	67.0	46.0	4.0	3.0
7	33.0	8.2	80.0	53.3	4.7	20.0	34.0	33.0	2.0	28.0
8	72.0	2.5	29.0	24.0	4.0	0.0	13.0	9.0	1.0	0.0
9	50.0	2.1	33.0	18.0	14.0	0.0	8.0	4.0	2.0	0.0
10	66.5	2.3	71.0	22.3	12.7	0.0	32.0	18.0	5.0	0.0
11	75.5	3.0	71.0	49.3	5.3	0.3	23.0	20.0	1.0	1.0
12	81.0	1.5	43.0	16.3	0.7	0.0	20.0	6.0	0.0	0.0
13	53.5	1.9	27.0	12.0	0.3	5.7	14.0	3.0	1.0	4.0
$\bar{x}$	45.5	6.3	60.5	37.5	7.7	2.4	32.0	20.6	2.6	3.3

[a]Primarily *F. roseum, F. moniliforme, F. oxysporum*, and *F. solani*.

Less deterioration occurred in samples (10-13) collected during October and November, when rainfall was less abundant. Low FFA content was significantly correlated with high seed germination (Table 2). An exception is sample 1, which had both low FFA content and low germination.

The moist preharvest period favored activity of seed-invading fungi. Frequency of isolation of fungi from whole seed ranged from 27.0 to 88.0%, and averaged 60.5% (Table 1). Incidence of embryo infection varied from 14.0 to 67.0%, and averaged 32.0%. Fungi were usually isolated from whole seed more often than from the embryo.

TABLE 2. Seed lot characteristics significantly correlated with fungal isolation

Seed lot characteristics and isolation of fungi			Correlation coefficient[a]
% Seed germination	x	% free fatty acids	–0.724
% Seed germination	x	total fungi in embryo	–0.611
% Seed germination	x	*Fusarium* spp. in embryo	–0.555
% Seed germination	x	*Diplodia gossypina* in embryo	–0.670
% Free fatty acids	x	total fungi in whole seed	+0.656
% Free fatty acids	x	*Fusarium* spp. in whole seed	+0.738
% Free fatty acids	x	total fungi in embryo	+0.856
% Free fatty acids	x	*Fusarium* spp. in embryo	+0.863

[a]A correlation coefficient greater than 0.532 is necessary for significance at the 5% level.

The most prevalent fungi isolated were *Diplodia gossypina* Cke., *Fusarium moniliforme* Sheldon, *F. oxysporum* Schlecht., *F. roseum* Lk. emend. Snyd. & Hans., *F. solani* (Mart.) Appel & Wr., and *Glomerella gossypii* Edg. *Fusarium* spp. were the most widely distributed and abundant, being detected in all 13 locations, and infecting an average of 37.5% of the whole seed and 20.6% of the embryos (Table 1). *Diplodia gossypina* was found in most locations, but usually colonized less than 10% of the whole seed and 5% of the embryos. *Glomerella gossypii* was isolated from five samples, and was detected in 2.4% of the whole seed and 3.3% of the embryos.

Other fungi detected infrequently were *Alternaria* spp., *Aspergillus* sp., *Chaetomium* spp., *Cladosporium* sp., *Curvularia* sp., *Helminthosporium* sp., *Mucor* sp., *Nigrospora* sp., *Olpitrichum* sp., *Paecilomyces* sp., *Penicillium* spp., *Phomopsis* spp., *Rhizoctonia solani* Kuehn, and *Tricothecium roseum* Lk. ex Fr.

The abundance of fungi in seed was generally associated with poor quality (Table 1). High levels of embryo infection by the total fungal population, *Fusarium* spp., and *D. gossypina*, were significantly correlated with low seed germination (Table 2). There was no significant relationship between germination percentage and fungal colonization of the whole seed. High quantities of FFA in the seed were significantly correlated with colonization of the whole seed and embryo by the total fungal population, particularly *Fusarium* spp.

Survival of field fungi in storage was studied in 7 commercially ginned seed lots. The samples were similar to their hand-ginned counterparts, averaging 49.8% germination and having an FFA content of 5.0%. Storage for ca. 5 months did not significantly reduce the mean germination of the seed lots (Table 3). The only significant change in survival of fungi took place in whole seed, where it averaged 15.0% less after storage. The mean embryo infection dropped by 5.1%. One sample, however, showed a

TABLE 3. Influence of storage at 15 C for 5 months on seed germination and fungus survival in commercially ginned samples[a]

	After harvest	After storage
% Germination	49.8 a	46.2 a
% Isolation from whole seed		
Fusarium spp.	36.1 a	22.3 b
Diplodia gossypina	10.5 a	9.0 a
Glomerella gossypii	2.2 a	1.4 a
Total fungi	59.1 a	44.1 b
% Isolation from embryo		
Fusarium spp.	13.0 a	9.5 a
D. gossypina	4.7 a	3.2 a
G. gossypii	0.8 a	1.5 a
Total fungi	23.8 a	18.7 a

[a]Row means followed by the same letter are not significantly different at the 5% level.

12.0% increase in fungal infection of the embryos during storage.

Fusarium spp. showed the only significant reduction in survival in whole seed, averaging 13.8% less after storage (Table 3). There was some variation in the survival of *Fusarium* spp. among seed lots, with little change evident in some cases, whereas the maximum reduction in one sample was 35.6%. Survival of embryo-infecting fungi was less affected by storage. A 3.5% decrease in the frequency of isolation of *Fusarium* spp. was the largest detected.

DISCUSSION.—During moist periods prior to harvest, fungi are afforded an opportunity to colonize seed in exposed, fluffed locks of cotton. We did not determine the exact time of fungal colonization of the seed. The frequent occurrence of infected seed in fluffed locks suggests, however, that limited invasion took place during boll opening, and increased during moist periods of field exposure. Extensive attack upon the fiber and seed during boll opening usually results in tight-lock, in which the fibers fail to fluff normally (6). Inasmuch as seed in fluffed locks are mechanically harvested, the weather during the period between boll dehiscence and harvest is considered a critical factor affecting seed deterioration and microbial activity.

Our findings, that reduced seed germination and high FFA content were associated with fungal invasion, agree with earlier reports (1, 2, 4, 7). Seed deterioration was detected, nevertheless, in several samples where fungal populations were relatively low, indicating that microorganisms were probably unimportant at some locations. Other factors influencing the relationship between the quantity of FFA and the frequency of seed invasion may have been (i) the lipolytic capabilities of the fungi involved (8); and (ii) the degree of fungal growth into the seed (9).

Increases in fungal populations detected in several lots indicated that some seed invasion may have occurred during storage. It is likely that the low moisture content of the seed lots restricted microbial activity. Consequently, sampling variation and multiple infection of individual seeds, making it difficult to identify the slower-growing fungi, may have affected the frequency of isolation.

Storage conditions favorable for preservation of seed viability were adequate for the survival of field fungi. Although there was a significant reduction of fungi in whole seed, the decrease in embryo infection was only slightly affected. Arndt (1) reported that *G. gossypii* was able to infect emerging cotton seedlings best when stored under conditions which favored survival of both the seed and fungus. As field fungi readily survive under these storage conditions until the following growing season, their presence in harvested seed constitutes the potential for a seedling disease problem.

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