# NonInterference of Pseudomonas in the Bright, Greenish-Yellow (BGY) Fiber-Fluorescence Test for Aspergillus flavus Boll Rot

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Mention of commercial products throughout this paper is for identification and does not constitute endorsement by USDA.

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ABSTRACT

Bright, greenish-yellow (BGY) fluorescence in raw cotton fiber is used to detect *Aspergillus flavus* boll rot. However, it was reported recently that *Pseudomonas* infection can also cause "yellow-to-green" fluorescence in cotton fiber in the field. Evidence indicates that, under the conditions used in our laboratory, *Pseudomonas* infection probably has caused few if any false positives in the BGY-fluorescence test. BGY-fluorescing fiber of commercial origin has been found consistently in our laboratory (1) to contain a fluorescing pigment chromatographically identifiable with a pigment produced by *A. flavus* on cotton fiber in pure culture and (ii) to be infected with *A. flavus* but not to be infected with *Pseudomonas*. In any case of doubt concerning recognition of the *A. flavus*-BGY-fluorescence, its identity can be checked quickly by the simple tests described here.

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Certain spots in raw cotton fiber (*Gossypium hirsutum* L.) with a bright, greenish-yellow (BGY) fluorescence were reported from this laboratory in 1955 to be caused by *Aspergillus flavus* [Lk.] Fr. (3). Infection was known to occur during or before the period of boll opening. Since that time, such fluorescing spots have been used as a detection device in surveying U.S. cotton of several crop years for *A. flavus* boll rot (4, 7, 8, 11, 13, 14). The fluorescence is associated with aflatoxins in the seeds at harvest (1, 5) and is currently being used by cottonseed processors in the rapid detection of seeds likely to contain aflatoxins. Recently, however, McCarter (9) reported that spots with "yellow-to-green" fluorescence can be caused in raw cotton in the field by infection with *Pseudomonas* sp. and has noted that "*Aspergillus flavus* is usually associated with similar spots." The basic capacity of many pseudomonads to produce a green-yellow fluorescence is well known (10, 15) and is not at issue here. The present paper is concerned rather with the more specific question of whether *Pseudomonas* infection leads to false positives in the BGY-fluorescence test to detect *A. flavus* boll rot as used in our laboratory.

Evidence reported in 1955 (3) supported the validity of the BGY-fluorescence test to detect *A. flavus* boll rot. Of a total of 116 BGY-fluorescing wisps of fiber from an equal number of bales of commercial cotton, 111 were clearly infected with *A. flavus*, a fungus not commonly observed in most cotton fiber at harvest. *A. flavus* was also found in BGY-fluorescing fiber in other samples from Arizona, Arkansas, California, and Texas (3, p. 1009). Of even more critical importance, the fluorescing pigment from field-infected fiber produced a single green-yellow fluorescing spot on a chromatogram and the $R_f$'s for pigments from fiber samples from different locations across the Cotton Belt matched each other and also matched the $R_f$'s for pigment from fiber incubated in pure culture with *A. flavus* (3, p.1011).

Evidence published since 1955 has reinforced our confidence in the BGY-fluorescence detection test for *A. flavus* boll rot. BGY-fluorescing fiber was examined for infection again in the crops of 1969 (13) and 1970 (11) and showed high levels of infection with *A. flavus*, whereas such infections were much less common in nonBGY-fluorescing fiber. Table 1 summarizes data on this point from the crop of 1970 (11, 12). In the Southeast, from which S. M. McCarter's samples were drawn, the fraction of BGY-fluorescing spots that was infected with *A. flavus* was very high (14/15), as in other areas, and the fraction of samples of nonBGY-fluorescing fiber that was infected with *A. flavus* from the same area was very low (4/186). Actually, the level of BGY-fluorescing spots has been extremely low in our surveys in the Southeast compared with that in other growing areas (4, 7, 8, 11, 13, 14), leaving little room for occurrence of false positives.

The BGY fluorescence has been traced to a substance derived from kojic acid, long known to be a

TABLE 1. Frequency of *Aspergillus flavus* infections in cotton fiber from spots with a bright, greenish-yellow (BGY) fluorescence, compared with such infections in fiber without the BGY fluorescence, crop of 1970

| | Fractions of samples with *Aspergillus flavus* infections | |
|---|---|---|
| Area | Fiber with BGY fluorescence | Fiber without BGY fluorescence |
| Southeast | 14/15 | 4/186 |
| Midsouth | 18/21 | 6/295 |
| Texas-Oklahoma | 70/78 | 26/264 |
| Western | 55/55 | 42/154 |

metabolite of *A. flavus* (6). In addition, a considerable degree of association has been found between the occurrence of the BGY fluorescence and that of aflatoxins (1, 5), now recognized as metabolites characteristic of *A. flavus*. The fact that the occurrence of BGY spots in cotton fiber has remained so nearly constant in geographical distribution (4, 7, 8, 11, 13, 14) seems to support the idea of a single causal agent for the observed fluorescence. Direct experiments to check on the possibility of any false positives from *Pseudomonas* infection in our data from across the Cotton Belt, however, are reported here.

MATERIALS AND METHODS. – Cultures of *Pseudomonas syringae* and *P. lachrymans*, both fluorescing pseudomonads, were supplied by R. W. Goth, USDA, Beltsville, Md. In addition, an isolate of *Pseudomonas* sp. was taken from a sample of fiber sent to us by S. M. McCarter on which a pure culture of the organism had been grown, and a second isolate was taken from a sample of cotton seed that he had incubated with a pure culture of the organism. Pure culture of the bacteria on cotton bolls was performed as previously described for *A. flavus* (3), except that incubation was in deep petri dishes with the bolls supported on glass beads over water and that room temp, approximately 23 C, was used instead of 30 C. King's Medium B (2) was used to detect fluorescent pigment production on agar, with incubation in 10-cm diam standard Pyrex petri dishes at room temp for 7 days. Observations for fluorescence were made, as in most of our previous studies, under a long-wave Blak-Ray ultraviolet lamp, model B-100A. The bulb used was a Westinghouse 100-w mercury flood lamp. Commercial cotton fiber consisted of classer's samples from Alex Hodgkins, USDA, Memphis, Tennessee.

Chromatographic separation of fluorescent pigments was done on 1-3 mg samples of BGY-fluorescing fiber. These were extracted with 0.1 ml of water and the extract was spotted onto the filter paper or thin-layer chromatographic (TLC) plate. The filter paper system included Whatman No. 1 filter paper, a developing solvent of 15% NaCl in water, and a 30-min development time. The TLC system included a 500-$\mu$m layer of Avicel on a glass plate, a solvent system containing formic acid/methyl ethyl ketone/tertiary butyl alcohol/ water (15/25/35/25), and a 2-h development time. The BGY spot is smaller in this system.

RESULTS. – Each of the four *Pseudomonas* cultures produced a yellow-to-green fluorescence when incubated on never-dried fiber in mature cotton bolls in pure culture. Further, wisps of fiber from these boll cultures, when dried and then incubated on King's Medium B, produced an outgrowth of bacteria onto athe agar with an accompanying greenish-yellow fluorescence in the agar.

In tests to detect *Pseudomonas* infection in commercial samples, wisps of BGY-fluorescing fiber were picked out of 1971 crop samples and examined for bacteria that produced fluorescent pigments. None of the wisps of BGY-fluorescing fiber from the commercial samples, however, produced any fluorescence in the agar, a result indicating that infections with fluorescing pseudomonads were not present. The 32 wisps came from bales (one bale per location, unless otherwise noted) ginned at Alamo, Texas; Arvin, Calif.; Brawley, Calif.; Casa Grande, Ariz., 2 bales; Clarksdale, Miss.; Commerce, Texas; Corpus Christi, Texas; Coyanosa, Texas; El Centro, Calif., 2 bales; Forney, Texas; Itasca, Texas; Lockhart, Texas, 2 bales; Millington, Tenn.; O Donnell, Texas; Parker, Ariz., 2 bales; Pearsall, Texas; Peoria, Ariz.; Queen Creek, Ariz., 3 bales; Safford, Ariz. San Joaquin, Calif.; San Juan, Texas; Terrell, Texas, 3 bales; Waco, Texas; and Woodsboro, Texas. A positive control sample of fiber that had been incubated previously in the never-dried condition with *Pseudomonas* sp., showed the presence of green-yellow fluorescence in the agar in this test, but no *A. flavus*. Of the same 32 wisps from 32 bales of commercial fiber, however, 25 developed an outgrowth of *A. flavus*. We assumed that the fungus

TABLE 2. Results of paper chromatography and Avicel thin-layer chromatography of water extracts from naturally infected bright greenish-yellow (BGY)-fluorescing fiber from the crop of 1971 in comparison with similar results for fiber incubated in pure culture with *Aspergillus flavus*

| Gin Location | Sample No. | $R_f$ for BGY-fluorescing spot on: Paper chromatogram[a] | Avicel thin-layer chromatogram[b] |
|---|---|---|---|
| Bishop, Tex. | 178-3 | 0.53 | 0.60 |
| Brawley, Calif. | 336-2 | 0.51 | 0.62 |
| Doddsville, Miss. | 113-3 | 0.53 | 0.60 |
| El Centro, Calif. | 344-2 | 0.51 | 0.62 |
| Itasca, Tex. | 196-1 | 0.53 | 0.61 |
| Leachville, Ark. | 080-2 | 0.53 | 0.60 |
| Mathis, Tex. | 173-3 | 0.53 | 0.60 |
| Parker, Ariz. | 302-1 | 0.52 | 0.62 |
| Pearsall, Tex. | 208-3 | 0.52 | 0.61 |
| Waco, Tex. | 200-1 | 0.52 | 0.62 |

[a] $R_f$ for spot from fiber incubated with *A. flavus* = 0.53

[b] $R_f$ for spot from fiber incubated with *A. flavus* = 0.61

had died in seven of the wisps; no other organism appeared consistently in these seven wisps.

Data on 1971 crop samples confirm our observations reported in 1955 that the chromatographic behavior of the pigment in naturally infected BGY-fluorescing fiber matches that produced by *A. flavus* on cotton fiber in pure culture (3). Ten samples consisting of ten wisps of BGY-fluorescing fiber, each from a different bale of commercial cotton, were extracted with water and the extract was chromatographed in two systems in comparison with a similar extract from cotton incubated in pure culture with *A. flavus* as previously described (3). The $R_f$ values obtained for the commercial fiber samples matched each other well and also matched the values for the extracts from the cotton incubated with *A. flavus* (Table 2). These chromatographic systems could be used conveniently by anyone wishing a confirmatory check on the cause of a suspect *A. flavus*-BGY spot in raw cotton fiber. In both of the above systems, fiber incubated with *Pseudomonas* and similarly treated failed to yield any yellow-fluorescing spot on the chromatogram, but did yield a purple-fluorescing spot of high $R_f$.

Cotton fiber incubated with *Pseudomonas* sp. exhibited certain characteristic changes in fluorescence when treated with acid or base, results quite different from those with *A. flavus*-incubated BGY fiber. Thus, when a small wisp of *Pseudomonas*-incubated fiber was placed into a depression on a white porcelain spot plate and was wetted with 1.0 N acetic acid, a salmon-colored fluorescence developed. The BGY fiber similarly treated showed a prompt loss of all fluorescence. *Pseudomonas*-incubated fiber treated with 0.1 N sodium hydroxide promptly developed a striking green fluorescence, whereas the BGY fiber again showed a complete loss of fluorescence.

DISCUSSION. – We find it impossible to state categorically that under some circumstances not known to us, *Pseudomonas* infection cannot cause false positives in the detection of *A. flavus* boll rot. On the other hand, we doubt very much that many false positives of this or any other origin have occurred in the work reported to date from our own laboratory. Variables in the ultraviolet light source might possibly be important. We have standardized on the Blak-Ray Model B-100A lamp. Differences in acuteness of visual perception by observers could possibly be involved. Out of eight observers in our own laboratory, however, six have shown no difficulty or hesitation in recognizing a characteristic type of fluorescing spot for which we have used the designation "BGY." With specific reference to *Pseudomonas*, we feel it possible that even if spots with a fluorescing pigment from *Pseudomonas* infection had been present in some of our samples, the instability of the fluorescence in preharvest weathering mentioned by McCarter (9) might have dulled their appearance under the ultraviolet lamp so greatly that we would not have recorded them as "BGY spots".

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