Distribution of Races of *Fusarium oxysporum* f. sp. *vasinfectum* Within the United States

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ABSTRACT

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Isolations of *Fusarium oxysporum* f. sp. *vasinfectum* inciting vascular wilt of cotton (*Gossypium hirsutum* L.) were collected from across the cotton belt in the United States. Fifty-three of these isolates that induced symptoms on artificially inoculated, greenhouse-grown cotton were also evaluated on two tobacco (*Nicotiana tabacum* L.) differentials. Eleven of the 53 isolates caused wilting on both Gold Dollar and Burley 5 tobacco, whereas 42 caused wilting only on Burley 5. Thus, these 11 isolates would be classified as race 2 of the fungus. Because the 11 isolates that caused wilting on both tobacco cultivars were derived from a wide geographical area (California, Alabama, South Carolina, and North Carolina), race 2 of the fungus is more prevalent and widespread than originally reported.

Six races of *Fusarium oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. have been identified. Race 1 is found across the cotton belt in the United States (2), in East Africa (7), and possibly in Italy (6), and race 2 is reported to occur in the United States at two sites in South Carolina (1). Race 3 occurs in Egypt (2), race 4 in India (2) and possibly the USSR (6), race 5 in the Sudan (8,9), and race 6 in Brazil and Paraguay (3,4). Race 2 has a wider host range than race 1; however, because of the believed limited distribution of race 2 and the fact that soybean and tobacco (which are used to differentiate races 1 and 2) are not immune to all isolates of race 1, Ebbels (7) suggested that race 2 be considered a variant of race 1.

Although race 1 of the fungus has been identified in a number of states in the United States, race 2 has only been reported at two sites in South Carolina. Most isolates of *F. oxysporum* f. sp. *vasinfectum* from the United States have not been classified. Identification of each isolate involves 1) isolation and identification of the fungus and 2) evaluation and comparisons of the host reactions of cotton, Gold Dollar flue-cured tobacco (*Nicotiana tabacum* L.), and Kentucky 5 burley tobacco or Yelredo soybean (*Glycine max* L.) after artificial inoculation.

The objectives of these experiments were to 1) isolate the fungus from cotton plants with vascular wilt disease that were collected from several areas of the United States, 2) determine the host reactions of two cottons and two tobacco differentials after artificial inoculation with these isolates, and 3) classify isolates as to race of *F. oxysporum* f. sp. *vasinfectum*.

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MATERIALS AND METHODS

Source of *Fusarium* isolates. Isolations were made from diseased cotton plants that showed Fusarium wilt symptoms. The plants were collected from three locations in South Carolina, three in North Carolina, two in Texas, and one in Alabama. Stem sections were cut from diseased stalks, surface-sterilized in 5% sodium hypochloride, rinsed in sterilized water, blotted dry, and plated on 1% water agar. After 2–3 days of growth, individual mycelial tips were transferred to acidified potato-dextrose agar. In addition to isolates from plant tissues, one isolate was taken from soil in a 30-yr-old regional Fusarium wilt test site. This isolate was recovered from a soil-dilution plate containing acidified potato-galactose agar (10). Pathogenicity of all isolates mentioned before (179) plus that of 22 wilt isolates from Mississippi, Missouri, and California was then evaluated on two cotton cultivars, Rowden and Stoneville 213. These two cultivars are known to be susceptible to races 1, 2, and 6 of *F. oxysporum* f. sp. *vasinfectum*. Because of the large number of cultures, evaluations were made over a series of experiments.

All but seven of the 201 isolates incited typical Fusarium wilt symptoms both on Rowden and Stoneville 213 after these cultivars were artificially inoculated with the individual isolates. Based on these results, the seven unknown isolates expressing atypical symptoms or avirulence on cotton were discarded. Only 53 isolates were retained for evaluations on tobacco. These were selected based on collection site, original culture, and/or cultivar from which the isolates were derived and consisted of 12 isolates from 3 locations in South Carolina; 12 from 3 locations in North Carolina; 3 from the vicinity of Brownfield, TX; 6 from Tallassee, AL; 5 from within about an 80-km radius of Stoneville, MS; 2 from Tulare and 4 from Kern counties in the San Joaquin Valley of California; 2 from unknown areas in California; and 7 from the Bootheel area of Missouri.

Cotton growth and inoculation. Evaluations of the resistance of cotton to each isolate were made on greenhouse-grown materials planted in rows spaced 15.2 cm apart on benches containing Wickham sandy loam soil. Two cotton seeds were planted in each of 10 preformed holes (6 × 32 mm) within a row. After planting, the soil was covered with brown paper kept moist until it was removed 3–4 days later. One week after planting, all rows were thinned to 10 individual plants spaced 5 cm apart within the row. At 15 days after emergence, the test plots were cultivated and the soil firmed around the plants. This operation raised the soil level around the plants by about 2 cm. Four weeks after planting, cotton plants were stem-inoculated by two injections per plant about 13 mm above the soil line following the technique described by Bugbee and Presley (5).

Tobacco growth and inoculation. Tobacco seeds were mixed with finely sifted soil and planted in flats containing steam-sterilized Wickham sandy loam soil covered with 1.3 cm of finely ground sphagnum moss. After seeding, the flats were covered with paper and kept moist until plant emergence. When the plants were about 3.8 cm tall, they were transplanted into 25.4-cm pots containing sterilized coarse sand. Plants were spaced 3.4 cm apart on the circumference of a circle 2.5 cm from the outer edge of each pot. Each pot contained 10 plants of Gold Dollar flue-cured and 10 plants of Kentucky 5 burley tobacco. These two tobaccos were used because they were reported to express a differential reaction to races 1 and 2 of *F. oxysporum* f. sp. *vasinfectum* by Armstrong and Armstrong (1). Each morning and afternoon from Monday through Friday, 500 ml of half-strength Hoagland's solution was added to each pot. On Saturdays and Sundays, pots were watered with tap water to flush any staling products. On extremely hot days, tap water was added to pots just before noon as needed to prevent wilting.

Tobacco plants were inoculated 10 days after transplanting. Plant roots were cut 2.5 cm from one side of the plant to a depth of 3.8 cm below the soil line. The inoculum, 150 ml per pot, was poured into the cuts and holes, which were covered with sand. One hour after inoculation, each pot was flushed by adding 700 ml of tap water. This technique is similar to that described by Armstrong and Armstrong (1).

Inoculum preparation and evaluation of host symptoms. Inoculum used to determine host reactions of both cotton and tobacco to the isolates studied was produced by growing isolates in flasks containing Czapek solution. These flasks were placed in a water-bath shaker maintained at 27 ± 0.5 C. After 7 days, each culture was mixed in a blender, spores were counted using a hemacytometer, and the inoculum was adjusted to a concentration of 2×10^6 microconidia per milliliter using sterilized distilled water. Evaluation of the pathogenicity of isolates and symptom expression of tobacco after inoculation with the unknown isolate were made relative to that of race 1 (ATCC 16421) and race 2 (ATCC 16611) of *F. oxysporum* f. sp. *vasinfectum*, which were known to be pathogenic to cotton.

After inoculation, plants of both tobacco and cotton that showed external symptoms of vascular wilt disease were removed at weekly intervals. Healthy plants remaining were counted 4 wk after inoculation. Wilting percentages were then calculated. Each test involving cotton as the host plant contained four replicates. Tests involving tobacco were replicated over time, with 10 plants of each cultivar evaluated per replicate.

Table 1. Race classification of isolates of *Fusarium oxysporum* f. sp. *vasinfectum* collected from across the United States, based on their reactions on two tobacco differentials

Isolate no.	Designation and/or source of origin	*Nicotiana tobacum*		Race classification
		Burley 5	Gold Dollar	
1	Indianola, MS	S[a]	R[a]	1
2	Stoneville, MS	R	R	1
3	Stoneville, MS	S	R	1
4	Stoneville, MS	S	R	1
5	Stoneville, MS	S	R	1
6	Old Pee Dee Stn., Florence, SC	S	R	1
7	Edisto Exp. Stn., Blackville, SC, on SC-1	S	R	1
8	Old Pee Dee Stn., Florence, SC, on SC-1	S	R	1
9	New Stn., Darlington Co., Florence, SC, on Pee Dee 875	S	R	1
10	New Stn., Darlington Co., Florence, SC, on SC-1	S	R	1
11	W-1-6, Hartsville, SC	S	R	1
12	W-1A-5, Hartsville, SC	S	R	1
13	W-1B-3, Hartsville, SC	S	S	2
14	W-1C-4, Hartsville, SC	S	S	2
15	W-1D-5, Hartsville, SC	S	R	1
16	W-1E-5, Hartsville, SC	S	S	2
17	W-1F-3, Hartsville, SC	S	S	2
18	G-2A-1, Laurinburg, NC	S	S	2
19	G-2-2, Laurinburg, NC	S	R	1
20	G-3-1, Laurinburg, NC	S	R	1
21	1-4, Tallassee, AL	S	R	1
22	A-22, Tallassee, AL	S	R	1
23	Id 1111-2, Tallassee, AL	S	S	2
24	0-4, Brownfield, TX	S	R	1
25	0-6, Brownfield, TX	S	R	1
26	CA 80-2, Tulare Co., CA	S	R	1
27	CA 80-4, Tulare Co., CA	S	R	1
28	CA 80-6, Kern Co., CA	S	S	2
29	CA 80-7, Kern Co., CA	S	R	1
30	CA 80-8, Kern Co., CA	S	R	1
31	CA 80-9, Kern Co., CA	S	R	1
32	MO-1, Bootheel area of MO	S	R	1
33	MO-2, Bootheel area of MO	S	R	1
34	MO-3, Bootheel area of MO	S	R	1
35	MO-4, Bootheel area of MO	S	R	1
36	MO-5, Bootheel area of MO	S	R	1
37	MO-6, Bootheel area of MO	S	R	1
38	MO-7, Bootheel area of MO	S	R	1
39	NCL-11, Raleigh, NC	S	R	1
40	NCL-12, Raleigh, NC	S	R	1
41	NCL-13, Raleigh, NC	S	R	1
42	NCL-23, Raleigh, NC	S	R	1
43	NCL-24, Raleigh, NC	S	R	1
44	NCL-26, Raleigh, NC	S	R	1
53	T-7, Tallassee, AL	S	S	2
54	T-6, Tallassee, AL	S	S	2
59	NCL-14, Raleigh, NC	S	R	1
60	NCL-15, Raleigh, NC	S	R	1
61	NCL-22, Raleigh, NC	S	R	1
62	R21-21, Tallassee, AL	S	R	1
63	T-8, Tallassee, AL	S	S	2
64	Race 1, ATCC 16421	S	R	1
65	Race 1, ATCC 16611	S	S	2
69	CA80-7, California	S	R	1
70	CA80-8, California	S	S	2

[a] S = susceptible, more than 50% of the plants wilted, and R = resistant, less than 50% of the plants wilted.

RESULTS AND DISCUSSION

Forty-two of the isolates tested and the ATCC 16421 check isolate did not cause wilt of Gold Dollar tobacco so they were classified as race 1 of *F. oxysporum* f. sp. *vasinfectum*; however, 11 of the final 53 isolates and the check, race 2 (ATCC 16611) of this fungus, incited wilt symptoms in Gold Dollar tobacco (Table 1). Four of these later isolates were derived from infected cotton from the vicinity of Hartsville, SC. This locality is one of two from which race 2 of the fungus was originally isolated in 1958 (1); however, none of the sites sampled in this study included the specific field from which race 2 was originally isolated. The other isolates of the fungus that caused wilting of Gold Dollar tobacco, and were thus classified as race 2, were derived from wilted cotton collected at Laurinburg, NC, from two different areas of California, and from infested soil and

infected plants collected at Tallassee, AL.

The isolates classified as race 2 because of their wider range of pathogenicity were derived from five different cottons; however, isolates of race 1 were derived from different plants of these same cotton lines. Thus, host specificity within cotton did not occur.

Although race 1 has been distinguished from race 2 based on the differential reaction of Gold Dollar tobacco after artificial inoculation, this host is not resistant to all isolates of race 1 (1,7). Thus, although 11 isolates in this study were identified as race 2, they may only be variants of race 1; however, because the 11 isolates showed a greater range in pathogenicity, caused severe wilting of Gold Dollar tobacco, and were obtained from a wide geographical area, I suggest that race 2 be considered a valid classification.

LITERATURE CITED

1. Armstrong, J. K., and Armstrong, G. M. 1958. A race of the cotton wilt Fusarium causing wilt of Yelredo soybean and flue-cured tobacco. Plant Dis. Rep. 42:147-150.
2. Armstrong, J. K., and Armstrong, G. M. 1968. American, Egyptian, and Indian cotton-wilt *Fusaria*. Their pathogenicity and relationship to other wilt *Fusaria*. U.S. Dep. Agric. Tech. Bull. 1219.
3. Armstrong, J. K., and Armstrong, G. M. 1978. A new race (Race 6) of the cotton wilt Fusarium from Brazil. Plant Dis. Rep. 62:421-423.
4. Armstrong, J. K., and Armstrong, G. M. 1980. Race 6 of the cotton-wilt Fusarium from Paraguay. Plant Dis. Rep. 64:596.
5. Bugbee, W. M., and Presley, J. T. 1967. A rapid inoculation technique to evaluate resistance of cotton to *Verticillium albo-atrum*. Phytopathology 57:1264.
6. Charodattan, R. 1969. Studies on strains of *Fusarium vasinfectum* Atk. I. On their morphology and pathogenicity on cotton. Proc. Indian Acad. Sci. (Sect. B) 70:139-156.
7. Ebbels, D. L. 1975. Fusarium wilt of cotton: A review, with special reference to Tanzania. Cotton Grow. Rev. 52:295-339.
8. Ibrahim, F. M. 1966. A new race of cotton wilt Fusarium in the Sudan Gezira. Emp. Cotton Grow. Rev. 43:296-299.
9. Ibrahim, F. M., and Khalifa, O. 1970. Fusarium wilt of cotton in the Gezira. Pages 189-194 in: Proc. Symp. Cotton Growth in the Gezia Environment. M. A. Siddig and L. C. Hughes, eds. Wad. Medani Republic Sudan Agric. Res. Corp.
10. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.