POPULATION STRUCTURE AND DYNAMICS AMONG FUSARIUM OXYSPORUM ISOLATES CAUSING WILT OF COTTON

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Abstract

From 1992 to 2015 nearly 3,000 isolates of *Fusarium* species from wilted cotton plants, seed, or cotton field soils were tested for pathogenesis using root-dip, stem-puncture, and soil-infestation assays. The greatest numbers of pathogens were identified by the root-dip assay. These were divided into vascular competent or root rot pathotypes using stem-puncture and soil-infestation assays, respectively. Populations within each pathotype were identified using vegetative compatibility tests and DNA sequencing. The vascular competent pathogens included more than 20 vegetative compatibility groups (VCGs). Isolates named races 1, 2, 6, and 8 occurred in VCG 0111, 0112, 0116, and CPRU 2, respectively. The root rot pathotypes race 3, race 4, and the Australian biotype occurred in VCGs 0113, 0114, and 01111, respectively. VCGs were tested for virulence to *Gossypium hirsutum* (seven cultivars), *Gossypium barbadense* 'Seabrook Sea Island 12B-2', *Gossypium arboreum* USDA Acc. No. A1-17, *Abelmoschus elegans* 'Clemson Spineless', *Medicago sativa* 'Grimm', *Nicotiana tabacum* 'Dixie Bright' and 'Gold Dollar', *Glycine max* 'Yelredo', *Cucumis melo* var. *cantalupensis* 'Magnum 45', and *Lycopersicon esculentum* 'Bonny Best'. All vascular competent VCGs had host ranges, and virulence to *Gossypium* species and cultivars, similar to those of the race 1 isolate ATCC 16421. VCGs of the root rot pathoype were specific to *Gossypium* species and were more virulent to several *G. barbadense* cultivars than race 1 isolates. Recent increases in severity of Fusarium wilt in California and Georgia were correlated with the appearance and spread of highly virulent strains of VCG 0114 and VCG CPRU 12, respectively.

Introduction

The fungus *Fusarium oxysporum* is a genetically diverse species composed of many genetically isolated populations. These populations can be identified by vegetative complementation tests as groups (VCGs) that have similar genetic makeup and, consequently, similar DNA sequences. In some cases a VCG shows a unique pattern of virulence to its host and is equivalent to a race, but in most cases VCGs do not vary in specificity of virulence and several may occur within a race (Correll, 1991). Puhalla (1985) showed that different formae speciales were made up of different VCGs and designated the cotton pathogen f. sp. *vasinfectum* as VCG 0110. Twelve VCGs (0111-01112) are now reported for f. sp. *vasinfectum* (Fernandez et al., 1994; Wang et al., 2010). In this study we used three bioassays to identify two pathotypes, and nit mutants to identify VCGs within pathotypes.

Methods and Materials

Vegetative compatibility testing and determination of DNA sequences were done using the protocols of Liu et al. (2011). VCGs were named according to the recommendations of Puhalla (1985) and Fernandez et al. (1994).

Root-dip assay: Plants were grown in a sandy loam/washed sand mix (3:1). Soil was removed from plants with two true leaves by submerging the soil mass in water. Roots were then placed in a 10^6 conidia/mL water suspension of the fungal isolate for 5 minutes and transplanted into a pasteurized (16 hours at 75°C) soil mix. Plants were incubated in controlled environment units programmed for 25°C, 13-hour days and 20°C, 11-hour nights.

Stem-puncture assay: Plants were grown in a greenhouse mix until the fourth true leaf was 2-3 cm wide. A single drop (20-30 µL) of 10^7 conidia/mL was formed on the tip of a 23-gauge needle and transferred to the surface of the hypocotyl 1-2 cm below the cotyledonary node. The needle, with the bevel side up, was placed through the drop into the hypocotyl to cut xylem vessels. Incubation was the same as for the root-dip assay.

Infested-soil assay: Seed were germinated in paper rolls for 24 hours at 30°C, followed by 24 hours at 20°C, and then transplanted into sandy loam/washed sand mix. One mL of $5x10^6$ conidia/mL was injected into each of six locations equidistant from each other, 1 cm away from the radicle, and 1-4 cm deep. Plants were incubated at 23°C, 13-hour days and 18°C, 11-hour nights.

Results and Discussion

From 1992 to 1995 we assembled more than 1,000 isolates of *Fusarium* resembling *F. oxysporum*. Of these isolates, 160 were virulent in both the root-dip and stem-puncture assays. No isolate was virulent in the infested-soil assay. Thus, all isolates were vascular competent. These isolates were assigned to 14 VCGs as shown in Tables 1 and 2. Where it could be ascertained, the VCGs were identified by the international code No. 011_. All other VCGs were identified by the USDA Cotton Pathology Research Unit (CPRU) number. VCG CPRU 1 was subdivided into A, B, and C subgroups; isolates in the A subgroup either failed to complement those in the C subgroup or gave very weak complements. Isolates in the B subgroup complemented isolates in all subgroups. For each group a reference isolate is given.

Table 1. Widespread vegetative compatibility groups of *Fusarium oxysporum vasinfectum*.

VCG	Reference Isolate	Total Isolates	Geographical Origin
0112	ATCC 16611*	35	AK(6), AL(15), SC(1)*, TX(11), China(2)
CPRU 1A	ATCC 24908	19	AL(7), CA(3), FL(2), LA(3), NC(1), MS(2), TX(1)
CPRU 1B	CPRU 84	23	AL(7), CA(2), GA(2), LA(2), NC(3), MS(4), SC(2), TX(1)
CPRU 1C	ATCC 24907	29	AK(8), AL(13), CA(2), LA(1), TX(5)
CPRU 2	NRRL 31665**	21	AK(8), AL (6), GA(1), LA(2), MS(9), SC(1)

* Reference isolate of race 2.
** An original isolate of race 8.

Table 2. Local vegetative compatibility groups of *Fusarium oxysporum vasinfectum*.

VCG	Reference Isolate	Total Isolates	Geographical Origin
0111	ATCC 16421	1	race 1 reference isolate, SC
0116	ATCC 36198	1	race 6 reference isolate, Brazil
CPRU 3	CPRU 241	11	Lafayette County, AK
CPRU 7	CPRU 977	3	Wilt Nursery, AL
CPRU 8	CPRU 1072	3	Wilt Nursery, AL
CPRU 9	CPRU 960	2	Wilt Nursery, AL
CPRU 10	CPRU 943	2	Wilt Nursery, AL
CPRU 11	CPRU MS 4-7-1	3	Deltapine Research Farm, MS
CPRU 12	CPRU 983	1	Wilt Nursery, AL
CPRU 13	CPRU 990	1	Wilt Nursery, AL
CPRU 14	CPRU 1082	1	Wilt Nursery, AL

In addition to the isolates in Tables 1 and 2, isolates CPRU 944, 947, 976, 989, 1070, and 1084 from the Wilt Nursery and isolate CPRU MS 4-9B from the Deltapine Research Farm were self-compatible but did not complement other isolates. Thus, we found a total of 21 VCGs.

The virulence of VCGs to *G. hirsutum* cultivars is shown in Table 3 and virulence to three different *Gossypium* species is shown in Table 4. There were no significant differences among VCGs in the specificity of their virulence to *Gossypium* species or cultivars. Consequently, all of these VCGs should be designated as race 1 following the criteria for race designation (Day, 1960). These VCGs also killed *A. elegans* and *M. sativa* but showed only weak and inconsistent virulence to *N. tabacum*, *G. max*, *C. melo*, and *L. esculentum*. All isolates within the 21 VCGs did significant damage to 'Rowden' in the stem-puncture assay. However, there were significant differences among isolates in each of the major VCGs in the level of virulence shown. Hence, assignment of an isolate to a VCG can be used as an indication of pathogenicity but it should not be used as an indicator of race. For example, races named 1,

2, 6, and 8 belong to four different VCGs and have different DNA sequences, but they do not show difference in virulence specificity to *Gossypium* species or cultivars. Thus, they should not be designated as different races.

Table 3. Mean leaf weight reduction[a] (% Control[b]) of *Gossypium hirsutum* cultivars caused by vegetative compatibility groups (VCGs) of *Fusarium oxysporum*.

		Cultivar							
VCG	No. Isolates	Rowden	Acala 44	STV 825	PM HS-26	Acala C-32	DP 50	DP 90	MEAN
0112	31	76.0	47.6	34.2	32.2	28.5	21.6	20.7	37.3
CPRU 1A	15	67.3	50.5	45.4	41.0	29.4	32.5	13.2	39.9
CPRU 1B	12	56.3	41.9	49.3	40.7	26.9	34.4	38.9	41.2
CPRU 1C	17	76.6	53.8	42.4	51.9	36.3	35.6	38.6	47.9
CPRU 2	7	50.3	48.4	41.0	41.3	34.9	25.9	28.6	38.6
CPRU 7-14	16	71.0	49.3	39.8	36.8	27.6	15.7	19.2	37.1
MEAN		66.3	48.6	42.0	40.7	30.6	27.6	26.5	40.3
Avirulent CKs	11	(7.5)[c]	12.8	(12.4)	11.1	1.8	2.3	(11.6)	(0.5)

a – Four or five replications per isolate; experiment repeated.
b – Plants puncture-inoculated with sterile water, 20 replications per cultivar.
c – ( ) = % greater than water control.

Table 4. Mean leaf weight reduction[a] (% Control[b]) of *Gossypium* species caused by vegetative compatibility groups (VCGs) of *Fusarium oxysporum*.

		Gossypium species[c]			
VCG	No. Isolates	*hirsutum*	*herbaceum*	*barbadense*	MEAN
0112	31	37.3	55.3	37.2	43.3
CPRU 1A	15	39.9	58.5	16.3	38.2
CPRU 1B	12	41.2	51.4	20.2	37.6
CPRU 1C	17	47.9	74.2	37.2	53.1
CPRU 2	7	38.6	59.2	25.1	41.0
CPRU 7-14	16	37.1	54.1	30.3	40.5
MEAN		40.3	58.8	27.7	42.3
Avirulent CKs	11	+0.5	+10.8	11.8	+0.3

a – Four or five replications per isolate; experiment repeated.
b – Plants puncture-inoculated with sterile water, 20 replications per cultivar.
c – *G. hirsutum* = mean of 7 cultivars; *G. herbaceum* = Acc. No A1-17 in the USDA Cotton Germplasm Collection; *G. barbadense* = Seabrook Sea Island 12B-2.

Isolates of *F. oxysporum* were obtained from the Fusarium wilt nursery again in 2010 and 2011 and tentatively identified as race 1 (12 isolates), race 2 (12 isolates), race 4 (12 isolates), or race 8 (9 isolates) by Amber Smith using elongation factor-1 DNA sequencing. These isolates were assigned to VCGs, and their virulence to 'Rowden' was determined (Table 5). Most of the isolates classified as race 2 and race 4, in fact, belonged to VCG CPRU 10 rather than VCG 0112 (race 2), or 0114 (race 4). Thus, the DNA sequences in this case were not reliable for VCG identification. The high frequency of VCG CPRU 10 is notable because only two isolates of it were found in the nursery in 1995. There were no appreciable differences in virulence among VCGs (Table 5).

Table 5. Frequency and virulence of vegetative compatibility groups (VCGs) found among *Fusarium oxysporum* isolates obtained in Alabama in 2010 and 2011[a].

VCG (race)	No. Isolates	F.D.I.[b] (%)	Leaf Weight Loss (%)	Shoot Weight Loss[c] (%)
0112 (race 2)	4	34	21	24
CPRU 1A (race 1)	4	27	9	6
CPRU 1B (race 1)	6	36	18	15
CPRU 2 (race 8)	9	35	18	19
CPRU 10	17	34	17	18
CPRU 13	1	35	20	21
CPRU 14	1	20	13	9

a – Cultures provided by A. Smith and K.S. Lawrence, Auburn University, AL.
b – Foliar disease index: % of half-leaf panels with symptoms.
c – Compared to plants inoculated with water, 3 weeks after inoculation.

In 2015 we determined VCG classification and virulence to 'Rowden' among 617 isolates obtained from 104 diseased plants from five counties in Georgia in 2014 (Table 6). Surprisingly, isolates of VCG CPRU 12, which previously had been found as only a single isolate in the wilt nursery in 1995 (Table 2), were recovered from all counties and 43 of 104 plants. VCG CPRU 12 isolates were slightly more virulent than those of VCGs CPRU 1B and CPRU 1C, the other prevalent groups (Table 6).

Table 6. Frequency and virulence of vegetative compatibility groups (VCGs) found among *Fusarium oxysporum* isolates obtained from infected plants in five counties of Georgia in 2014[a].

VCG (race)	No. of Isolates	Leaf Weight Loss (%)[b]
0112 (race 2)	16	29
CPRU 1B (race 1)	107	33
CPRU 1C (race 1)	26	32
CPRU 2 (race 8)	2	37
CPRU 10	11	20
CPRU 12	114	37
CPRU 22[c]	9	25

a – Infected stems provided by R.C. Kemerait, Jr., University of Georgia.
b – Compared to plants inoculated with avirulent strains, 3 weeks after inoculation.
c – Reference isolate CPRU GA 2-1-6.

In 2015 we obtained 17 isolates of race 4 from California (R.M. Davis, University of California, Davis) and 23 isolates of *F. oxysporum* from diseased tissue and cultures provided by A. Gu, Xinjiang Agricultural University, Xinjiang, China. These isolates were compared with known race 4 isolates maintained by the USDA Northern Regional Research Laboratory (NRRL). All 17 isolates from California, 19 of the 23 from China, and all NRRL isolates belonged to VCG 0114. Race 4 isolates from California strongly complemented VCG 0114 isolates from China, where they are called race 7 (Figure 1).

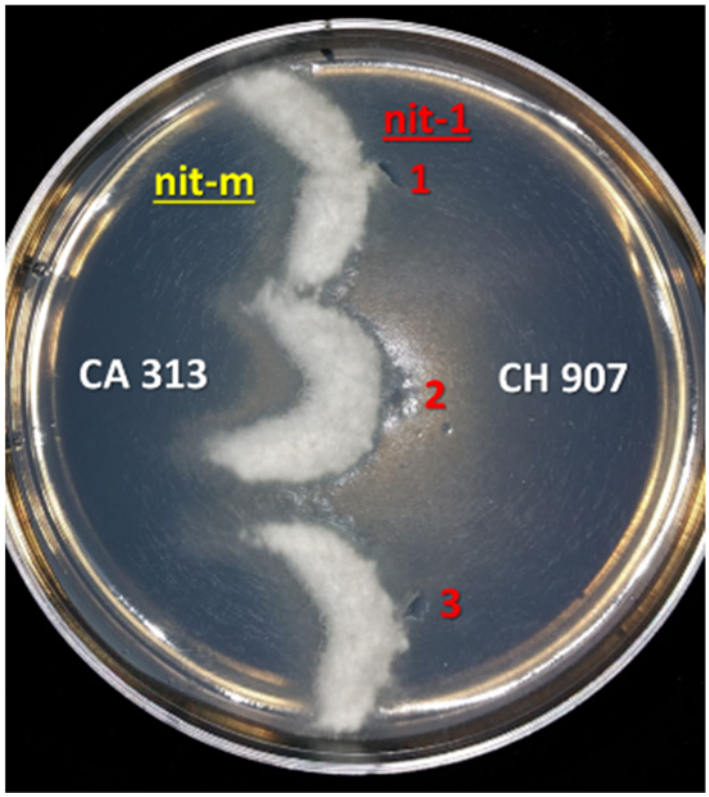

Figure 1. Complementation of VCG 0114 isolates from California and China.

Complementation of California or Chinese isolates with NRRL isolates was obvious but not as strong as between California and Chinese isolates alone. This indicates that VCG 0114 isolates in California and China are closely related and not different races. Isolates from both areas attack *G. hirsutum* as well as *G. barbadense* cultivars. Since all isolates complement the reference isolate ATCC 16613 of race 4 from India, they should collectively be referred to as race 4 or VCG 0114. We have not found VCG 0114 in the US outside of California.

All isolates of race 3 belong to VCG 0113 (Katan and Katan, 1988). DNA sequences have shown that isolates CA-3 and CA-11 are similar, but not identical, to the reference isolate of race 3, ATCC 16612. We are currently studying the distribution, if any, of VCG 0113 in the US.

All isolates from the US have been tested for complementation with four isolates of VCG 01111 from Australia. VCG 01111 was not found in the US.

Isolates in VCGs 0113, 0114, and 01111 cause severe disease in the soil-infestation but not the stem-puncture assay. Therefore, we designate them collectively as the root rot pathotype. They generally do not invade the shoot for several weeks after inoculation and then enter through the pith tissue and older xylem vessels. Invasion of leaves may not occur until plants are severely wilted. Isolates of this pathotype also cause more severe disease in *G. barbadense* cultivars, such as Pima S-2 and Pima S-7, than do race 1 isolates. These isolates occur in heavy loam or clay soils at neutral or alkaline pH and can be devastating without nematodes. Consequently, they are a great threat to cotton production in many areas of the US where Fusarium wilt is not currently a problem.

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