Selection for *Fusarium oxysporum* f. sp. *niveum* Race 2 in Monocultures of Watermelon Cultivars Resistant to Fusarium Wilt

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Florida Agricultural Experiment Station Journal Series paper R-01257.
Accepted for publication 8 October 1991 (submitted for electronic processing).

ABSTRACT

Hopkins, D. L., Lobinske, R. J., and Larkin, R. P. 1992. Selection for *Fusarium oxysporum* f. sp. *niveum* race 2 in monocultures of watermelon cultivars resistant to Fusarium wilt. Phytopathology 82:290-293.

In long-term monocultures of different watermelon cultivars, Fusarium wilt incidence was as high in wilt-resistant cultivars as in wilt-susceptible ones. Isolates of *Fusarium oxysporum* f. sp. *niveum* obtained from wilted resistant cultivars were predominantly the highly aggressive race 2, whereas isolates obtained from susceptible cultivars were predominantly race 1. To determine whether these cultivars also influenced populations of the pathogen in soil, Florida Giant, the highly susceptible cultivar, was used as a trap host to recover *F. o. niveum*. Races were determined by vegetative compatibility grouping. When steam-sterilized soil and various proportions of race 1 and race 2 were mixed, the races were recovered from wilted Florida Giant in the same proportions added to soil. Thus, this method is suitable for determining the proportion of *F. o. niveum* in the soil that is race 2. In monoculture plots of the race 1-resistant Calhoun Gray and Dixielee cultivars, race 2 constituted over 70% of the pathogen population in the soil. In plots of the susceptible Florida Giant cultivar, race 2 composed less than 10% of the soil population of the pathogen. Race 1-resistant cultivars selectively increased race 2 of *F. o. niveum* in soil.

Fusarium oxysporum Schlechtend.:Fr. f. sp. *niveum* (E. F. Sm.) Snyd. & Hans., the cause of Fusarium wilt of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), is widespread in the watermelon-producing regions of the world and limits production in many areas. In Florida, resistant cultivars and a long rotation are the primary control strategies used by growers (6).

F. o. niveum has been separated into two or three pathogenic races based on virulence on cultivars that differ in levels of resistance to Fusarium wilt (3,5,14). Two races were described in Florida; race 0 wilted only susceptible cultivars, and race 1 wilted 100% of susceptible cultivars and caused some wilt in moderately resistant cultivars such as Charleston Gray (5). However, differences among strains of the pathogen may not be great enough to distinguish between races 0 and 1 (1,10). A highly aggressive race capable of causing severe wilt in all cultivars tested was described in Israel in 1976 and was designated as race 2 (14). Race 2 has also been found in Texas (11), Oklahoma (2,12), and Florida (10,12).

Watermelon cultivars are generally described as resistant or susceptible to *F. o. niveum*, but actually range continuously from susceptible to highly resistant (6,13). Crop rotation is necessary for disease control even when highly resistant cultivars are grown (6,7). After 4–5 yr of a cultivar monoculture test, the race 1-resistant cultivars Calhoun Gray and Dixielee had the same wilt incidence as the race 1-susceptible cultivars Florida Giant and Sugar Baby (7,8). This loss of expressed resistance was not related to higher populations of *F. o. niveum* because propagule counts were similar for the resistant and susceptible cultivars (300–400 propagules per gram of soil). It was speculated that there could have been a population shift in the soil in these monoculture plots to highly aggressive strains of the pathogen that are not controlled by the resistance genes of these cultivars.

F. o. niveum is normally distinguished from other formae speciales and saprophytic strains of *F. oxysporum* by testing for pathogenicity to watermelon. This is laborious and time-consuming, and usually <30% of the strains from the soil are *F. o. niveum* (D. L. Hopkins, *unpublished*). Races of *F. o. niveum* are normally distinguished by pathogenicity tests on differential cultivars (3,11,14). Race 2 of *F. o. niveum* can also be distinguished from race 1 with vegetative compatibility tests (10). Vegetative compatibility in *F. o. niveum* appears to be one of the least complex of the formae speciales that have been studied, allowing it to be useful for identification of race 2. In some formae speciales, vegetative compatibility is too complex to be useful in race determinations.

The purpose of this study was to determine the effect of monocultures of watermelon cultivars with different levels of resistance to Fusarium wilt on the proportion of race 2 strains of *F. o. niveum* in the soil. To facilitate this study, a technique was developed whereby the highly susceptible cultivar Florida Giant was used as a trap host to selectively recover *F. o. niveum* from soil.

MATERIALS AND METHODS

Monoculture of watermelon cultivars. Ten watermelon cultivars representing a continuum of resistance to race 1 of *F. o. niveum* (6,13) were grown in long-term monoculture beginning in 1979 and continuing to the present as previously described (7,8). The 10 cultivars, from most resistant to most susceptible, were Smokylee, Calhoun Gray, Dixielee, Sugarlee, Crimson Sweet, Charleston Gray, Jubilee, Sugar Baby, Congo, and Florida Giant. Plots were marked by permanent posts so that each cultivar could be grown in the same plots each year. The cultivars used in this study were Calhoun Gray and Dixielee, both highly resistant; Crimson Sweet and Charleston Gray, both moderately resistant; and Florida Giant, susceptible. The experiments were conducted with soil and *F. o. niveum* isolates collected after 5–10 yr of monoculture.

Greenhouse bioassay of Fusarium wilt. Soil from cultivar monoculture plots was brought into a greenhouse and placed in 10-cm-diameter pots. Treatments, consisting of cultivars planted, were replicated four times with two pots per replicate. Ten seeds were planted per pot. Greenhouse temperatures ranged from 22 to 30 C during the bioassays. Wilted seedlings were counted and removed daily for 5 wk after planting, and the percentage of plants wilted was calculated. The experiment was conducted twice.

Fusarium isolates. Isolates of *F. o. niveum* were obtained from surface-sterilized hypocotyl sections from wilted seedlings. Hypocotyl sections were plated on Komada's Fusarium-selective medium (9). Soil isolates were obtained by dilution-plating soil suspensions on Komada's medium and selecting *F. oxysporum* from the plates by characteristic growth and morphology after 7–10 days. Single-spore cultures of each isolate were stored in soil tubes.

Pathogenicity and race determinations on differential cultivars. To simulate natural conditions, chlamydospores of *F. o. niveum* were used as inoculum. Chlamydospores were produced by the addition of a thoroughly mixed 7-day-old culture in liquid medium (14), which consisted of microconidia and chopped mycelia, to autoclaved soil (Apopka fine sand). The soil was moistened, mixed, and air-dried for 4–8 wk. Microscopic examination after staining with trypan blue lactophenol revealed that fusarium propagules that remained viable in dried soil were primarily chlamydospores (>90%). The number of propagules per gram of soil was determined for each isolate by dilution plating. These stock soils (containing 10^4–10^5 chlamydospores per gram of soil) were mixed with the soil to be infested to produce the desired inoculum level, which was 100 ± 20 chlamydospores per gram of soil.

Pathogenicity tests were conducted using the susceptible cultivar Florida Giant to separate isolates of *F. o. niveum* from nonpathogenic isolates. In virulence tests to determine race designations for isolates of *F. o. niveum*, the cultivars Florida Giant, Charleston Gray, Crimson Sweet, and Calhoun Gray were used. Seeds were planted directly in artificially infested soil (100 ± 20 chlamydospores per gram of soil). Four replicate pots (10-cm-diameter) of 10 plants each were used for each treatment. Greenhouse temperatures ranged between 22 and 30 C during the tests. Wilted seedlings were counted and removed three times weekly for 4 wk, and the cumulative percentage of wilt was calculated. Isolates were considered race 2 if high and equal disease incidence (>50%) occurred in all four cultivars. Isolates were considered race 1 if they caused less disease in Calhoun Gray (<50% disease) than in the other three cultivars (>50% disease). All pathogenicity tests for race determinations were run two or more times per isolate unless the results were near the threshold for separating race 1 from race 2 (40–60% wilt in Calhoun Gray); these isolates were subjected to a third test.

Use of highly susceptible watermelon as a trap plant to selectively recover *F. o. niveum* from soil. Florida Giant was planted either in steam-sterilized soil to which known proportions of isolates of the three vegetative compatibility groups (VCG 0080, race 1; VCG 0081, race 1; and VCG 0082, race 2) had been mixed to give a total of 100 propagules of *F. o. niveum* per gram of soil or in test soil from the cultivar monoculture plots. When Florida Giant seedlings wilted, *F. o. niveum* was isolated from hypocotyl sections of the seedlings. Isolates were then tested for vegetative compatibility grouping as described below. These experiments were conducted twice.

Vegetative compatibility grouping. Previously described methods were used (4,15). Nitrate-nonutilizing (*nit*) mutants of the *F. o. niveum* isolates were obtained on potato-dextrose agar containing 1.5% $KClO_3$. Two pairs of complementary *nit* mutants from each of the three distinct VCGs of *F. o. oxysporum* were selected for use as tester strains (10). Each tester pair consisted of a Nit M and a *nit* 1 or *nit* 3 phenotypic mutant as defined previously (4). A multiple cross-plating technique permitted complementation tests with six different unknowns against seven tester strains (10). Complementation tests were conducted two or more times with each unknown.

RESULTS

Bioassay of cultivar monoculture soil for Fusarium wilt. In soil from Florida Giant and Charleston Gray monoculture plots, the incidence of wilt among the five cultivars corresponded with reported resistance to race 1 of *F. o. niveum* (Table 1) (6,13). The least wilt occurred in the highly resistant Calhoun Gray and Dixielee. However, in soil from the Crimson Sweet, Calhoun Gray, and Dixielee plots, the amount of wilt was similar among all five cultivars regardless of their resistance levels to race 1. When the test was repeated a month later, the amount of wilt in soil from these same three monoculture plots was again similar in all five cultivars (Crimson Sweet soil = 12–26% wilt, Calhoun Gray soil = 21–30% wilt, and Dixielee = 22–30% wilt); whereas, in soil from Florida Giant and Charleston Gray monoculture plots, wilt incidence corresponded with known levels of resistance of the cultivars to race 1 of *F. o. niveum*.

Pathogenicity and race determination of isolates of *F. o. niveum* obtained from wilted plants. To determine why the Fusarium wilt resistance of Calhoun Gray was no longer effective in monoculture, the race of *F. o. niveum* isolates obtained from wilted

TABLE 1. Fusarium wilt incidence among watermelon cultivars grown in cultivar-monoculture soils

	Wilt incidence per cultivar (%)[y,z]				
Monoculture soil[x]	FG	CG	CS	Cal. G	DL
Florida Giant	49 c	33 bc	35 bc	14 a	16 ab
Charleston Gray	60 b	41 ab	53 b	28 a	29 a
Crimson Sweet	13 a	11 a	25 a	8 a	24 a
Calhoun Gray	42 a	34 a	42 a	39 a	30 a
Dixielee	39 a	32 a	35 a	24 a	29 a

[x] Soil was collected from plots that had been planted in a monoculture of the designated cultivar for 5 yr. The *Fusarium oxysporum* f. sp. *niveum* populations in these soils ranged from 300 to 400 propagules per gram of soil.

[y] FG = Florida Giant, CG = Charleston Gray, CS = Crimson Sweet, Cal. G. = Calhoun Gray, and DL = Dixielee.

[z] Average percentage of plants that died of Fusarium wilt within 5 wk of planting in monoculture soil in the greenhouse. Means in rows followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test; data were analyzed after transformation to arc sine $\sqrt{x}$.

TABLE 2. Race distribution of isolates of *Fusarium oxysporum* f. sp. *niveum* isolated from wilted watermelon seedlings in the cultivar-monoculture plots

	5-yr monoculture[y]		8-yr monoculture[y]	
Cultivar[x]	Total isolates	Proportion of race 2	Total isolates	Proportion of race 2
Florida Giant	20	0.05(0,0.25)[z]	23	0.17(0.05,0.40)
Charleston Gray	20	0.30(0.12,0.54)	18	0.39(0.08,0.58)
Crimson Sweet	11	0.45(0.17,0.77)	15	0.53(0.27,0.79)
Calhoun Gray	9	0.67(0.35,0.93)	20	0.95(0.51,0.91)

[x] Strain of *F. o. niveum* were isolated from wilted plants of these cultivars grown in a monoculture. The experiment was conducted after 5 yr and after 8 yr of cultivar monocultures.

[y] The race designations of the *F. o. niveum* strains were determined by inoculation of differential watermelon cultivars.

[z] Values in parentheses are the confidence intervals for the proportions ($P = 0.95$).

plants was determined for four of the cultivar monocultures 5 yr after the monoculture test began, and the test was repeated 3 yr later (Table 2). The predominant race obtained from wilted Florida Giant was race 1 in both years. The proportions of race 1 and race 2 obtained from wilted Charleston Gray and Crimson Sweet varied considerably, with race 2 being obtained more frequently than it was from Florida Giant. In Calhoun Gray monoculture plots, *F. o. niveum* race 2 was isolated more frequently from wilted plants than race 1, especially after 8 yr of monoculture.

Pathogenicity and race determination of soil isolates of *F. o. niveum*. Isolates of *F. oxysporum* were obtained by dilution-plating of soil suspensions from the various monoculture plots on Komada's medium. Fifty-five isolates were obtained from Florida Giant monoculture plots and fifty isolates each from Crimson Sweet and Calhoun Gray plots (Table 3). Eighteen, 16, and 17 of the *F. oxysporum* isolates from these monoculture plots were pathogenic to watermelon. These *F. o. niveum* isolates were predominantly race 1 when recovered from Florida Giant and Crimson Sweet monoculture soil, but more than two-thirds of the isolates obtained from Calhoun Gray monoculture soil were race 2.

In a repeat of the test 2 yr later, the trends were similar with eight of 50 *F. oxysporum* isolates from the Florida Giant monoculture soil being *F. o. niveum*, and all of these were identified as race 1. There were also eight *F. o. niveum* isolates of 50 total from the Crimson Sweet soil, and two of these were race 2. Of 50 isolates from the Calhoun Gray monoculture soil, 11 were *F. o. niveum* and eight of these were race 2.

Use of Florida Giant as a trap plant and vegetative compatibility tests to determine the proportion of race 2 in soil. Using Florida Giant as a trap host to selectively recover *F. o. niveum* race 1 and 2 from soil, the proportions of VCG 0080 (race 1), VCG 0081 (race 1), and VCG 0082 (race 2) isolates recovered from wilted seedlings were very similar to the proportions originally mixed into the soil. The recovered ratio of race 1 to race 2 was similar to the known ratio originally mixed in the soil (Table 4). In the second test, recovered ratios of race 1 to race 2 were again very similar to the known ratio (known ratio 2:1, observed 16:7; known 9:1, observed 22:3; known 1:4, observed 6:19).

Florida Giant seedlings were used to recover *F. o. niveum* from cultivar monoculture soils and vegetative compatibility tests distinguished race 1 from race 2 in two separate tests conducted 6 mo apart (Table 5). Race 1 was found to compose over 90% of the soil population of *F. o. niveum* at least in Florida Giant monoculture plots; whereas, race 2 accounted for at least 75% of the *F. o. niveum* in the Calhoun Gray and Dixielee plots. With Crimson Sweet and Charleston Gray monoculture plots, race 1 was found to make up two-thirds to three-fourths of the soil population of *F. o. niveum*.

TABLE 3. Pathogenicity and race distribution of isolates of *Fusarium oxysporum* obtained from soil in the cultivar-monoculture plots[y]

Monoculture soil	Total *F. oxysporum* isolates	Number of *F. o. niveum* isolates	Proportion of race 2
Florida Giant	55	18	0.06(0,0.26)[z]
Crimson Sweet	50	16	0.06(0,0.31)
Calhoun Gray	50	17	0.71(0.43,0.89)

[y] *F. oxysporum* isolates were selected by characteristic growth on Komada's medium (9), and *F. o. niveum* isolates were identified by pathogenicity to susceptible watermelon. Races were determined by pathogenicity to differential cultivars.

[z] Values in parentheses are the confidence intervals for the proportions ($P = 0.95$).

DISCUSSION

In the fifth year of a cultivar monoculture test, wilt incidence in Dixielee and Calhoun Gray, both highly resistant to race 1 of *F. o. niveum*, was equal to that of highly susceptible cultivars (8). The loss of resistance in Dixielee and Calhoun Gray grown in a monoculture was not the result of higher populations of *F. o. niveum*, because the plots contained populations of the pathogen similar to that in monoculture plots of the highly susceptible Florida Giant (300–400 propagules per gram of soil). In this study, cultivars with different levels of resistance to *F. o. niveum* race 1 had similar levels of wilt when planted in Dixielee and Calhoun Gray monoculture soil. However, the same cultivars planted in Florida Giant and Charleston Gray monoculture soil had different levels of wilt that corresponded to published resistance rankings (6,13). All cultivars had similar, low levels of wilt in soil from the Crimson Sweet monoculture plots, but this has been reported to result from induced soil suppressiveness (8). We hypothesized that the loss of resistance to Fusarium wilt in monoculture plots of Dixielee and Calhoun Gray resulted from the selection for strains of *F. o. niveum* that overcame the resistance genes of these cultivars.

In support of this hypothesis, *F. o. niveum* isolates obtained from wilted Calhoun Gray plants in the monoculture plots were mostly race 2, and none of the commercial watermelon cultivars have genes for resistance to this race. In contrast, isolates obtained from wilted Florida Giant plants were mostly race 1, and race 2 was obtained infrequently. Soil populations of race 1 and race 2 in the cultivar monoculture plots reflected similar trends. Total *F. o. niveum* populations in Calhoun Gray and Florida Giant plots were nearly identical (8). However, the proportions of race 1 and race 2 in these populations varied significantly among the cultivars. Race 1 constituted more than 90% of the total population in Florida Giant plots; whereas, race 2 was the predominant race in Calhoun Gray and Dixielee plots. Apparently, a monoculture of cultivars highly resistant to race 1 of *F. o. niveum* selectively increased the proportion of race 2 in the soil.

The use of Florida Giant to selectively recover *F. o. niveum* from soil coupled with vegetative compatibility assays to distinguish race 1 from race 2 was a more convenient and cost-effective method for determining proportions of race 1 and race 2 populations in the soil than was pathogenicity and differential host ranges. This technique was based on the assumption that Florida Giant was highly susceptible to both races, so the proportion of plants infected with race 1 or race 2 would be proportional to the levels of race 1 and race 2 in the soil. This pro-

TABLE 4. Use of Florida Giant as a trap plant along with vegetative compatibility to determine the ratio of *Fusarium oxysporum* f. sp. *niveum* race 1 to race 2 in soil

Proportions added (VCG 0080:VCG 0081:VCG 0082)[y]	Observed frequency[z]		Expected ratio	Chi-square	*P* value
	Race 1	Race 2			
1:1:1	17	8	2:1	0.02	0.9
8:1:1	23	2	9:1	0.11	0.7–0.8
1:8:1	26	2	9:1	0.26	0.5–0.7
1:1:8	7	18	1:4	1.00	0.3–0.5

[y] Proportions of VCG 0080 (race 1), VCG 0081 (race 1), and VCG 0082 (race 2) isolates of *F. o. niveum* were mixed into sterile soil to provide an inoculum concentration of 100 propagules of *F. o. niveum* per gram of soil.

[z] Florida Giant watermelon were planted in the soil mixes, and *F. o. niveum* isolates were obtained from wilted plants. Nitrate-nonutilizing mutant tester strains were used to determine the ratio of race 1 isolates (VCG 0080 + VCG 0081) to race 2 isolates (VCG 0082) recovered from the wilted plants.

TABLE 5. Proportion of the soil population of *Fusarium oxysporum* f. sp. *niveum* that is race 2 in cultivar-monoculture plots

Monoculture plots	Recovered isolates: Proportion of race 2 (VCG 0082)[y]
Experiment 1	
Florida Giant	0.02 (0, 0.11)[z]
Crimson Sweet	0.22 (0.09, 0.42)
Calhoun Gray	0.75 (0.58, 0.86)
Experiment 2	
Florida Giant	0.08 (0.01, 0.26)
Charleston Gray	0.36 (0.18, 0.57)
Crimson Sweet	0.36 (0.18, 0.57)
Dixielee	0.96 (0.80, 1.00)
Calhoun Gray	0.96 (0.80, 1.00)

[y] Florida Giant watermelon were planted in soil from the various monoculture plots, and *F. o. niveum* isolates were isolated from wilted plants. Proportions of the various vegetative compatibility groups of the isolates were determined using nitrate nonutilizing tester strains. Total soil populations of *F. o. niveum* were 300–400 propagules per gram of soil. Experiment 1 was conducted with soil collected in January, and experiment 2 was run 6 mo later.

[z] Values in parentheses are the confidence intervals for the proportion ($P = 0.95$).

cedure eliminated the need to initially separate *F. o. niveum* isolates from nonpathogenic *F. oxysporum* isolates via pathogenicity tests, and, when used with previously described vegetative compatibility grouping assays (10), proportions of *F. o. niveum* race 1 and race 2 isolates in the soil could be determined more quickly, accurately, and more economically than with inoculation tests in the greenhouse.

Repeated monoculture of cultivars that have a high-level of wilt resistance to *F. o. niveum* race 1 increased the levels of race 2 in the soil. This could mean that race 2 was already present at low levels in the soil before these experiments or that race 2 was introduced on infested seed (11). The first explanation is supported by isolation of race 2 as early as 1970 in Florida (10). This would explain the requirement for long rotations for even highly resistant cultivars.

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