Identifying Races of *Fusarium oxysporum* f. sp. *niveum* in South Carolina Recovered From Watermelon Seedlings, Plants, and Field Soil

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Abstract

Fusarium wilt of watermelon (*Citrullus lanatus*), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *niveum*, is the most serious disease of watermelon in South Carolina and other southeastern U.S. states. Isolates of *F. oxysporum* collected from field-grown plants, greenhouse-grown seedlings, and field soil between 1999 and 2018 were inoculated onto three differential watermelon cultivars to identify races. Of 197 isolates obtained from plants, 12% were nonpathogenic, 2% were race 0, 23% were race 1, and 63% were race 2. One collection of isolates from greenhouse seedlings was exclusively race 1 and the other was exclusively race 2. Seventeen of 81 soil isolates were pathogenic: five were race 1 and 12 were race 2. Reactions of *C. amarus* PI 296341-FR, Carolina Strongback, and SP-6,

cultigens with resistance to race 2, did not differ significantly among five highly virulent race 2 isolates and a standard race 2 isolate, indicating a lack of a race 3 phenotype. Forma specialis-specific primers matched phenotypic race identification for 74% of the isolates. Race-specific primers based on a secreted-in-xylem elicitor present in race 0 and 1 isolates matched phenotypic race identification for 66% of the isolates. Because a majority of the *F. oxysporum* f. sp. *niveum* isolates from South Carolina were race 2, integrated management practices should be used until commercial cultivars with resistance to race 2 are available.

Keywords: fungi, vegetables, etiology

Fusarium wilt of watermelon (*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai), caused by *Fusarium oxysporum* Schltdl.:Fr. f. sp. *niveum* (FON), is one of the most serious diseases of watermelon worldwide (Martyn 2014, 2017). *Fusarium* wilt can significantly reduce the number of watermelon fruit per hectare, the total weight of fruit harvested per hectare, and the size of individual fruit (Everts and Hochmuth 2011; Keinath and Hassell 2014b; Keinath et al. 2019). A large percentage of fruit may not reach the minimum marketable weight, 4.5 kg for triploid fruit and 6.2 kg for diploid fruit. For example, only 12% of the fruit produced by a susceptible triploid cultivar were marketable size in heavily infested fields in Georgia and South Carolina (Keinath et al. 2019).

As in other *Fusarium* wilt pathosystems, races of FON are defined based on symptoms caused on differential cultivars that possess a gene or genes conferring resistance to different races. Based on the current set of four differential *Citrullus* cultivars for *Fusarium* wilt of watermelon, four races of FON, designated 0, 1, 2, and 3, can be distinguished (Martyn 2017). The differential cultivars Sugar Baby, Charleston Gray, and Calhoun Gray are inoculated if races 0, 1, and 2 are suspected among a collection of isolates (Zhou and Everts 2003). If race 3 isolates are suspected, then *C. amarus* Schrad. (syn. *C. lanatus* var. *citroides*) plant introduction (PI) 296341-FR, a *Fusarium* wilt-resistant selection of PI 296341 with resistance to race 2, is included (Bruton et al. 2010; Martyn 2017; Zhou et al. 2010). One problem with using the differential cultivar set is that seed of Calhoun Gray and PI 296341-FR is not available commercially. Zhou and Everts (2003) considered Calhoun Gray, followed by

Dixielee and Allsweet, to be the most effective cultivars for separating races 1 and 2.

Races 0, 1, and 2 are distributed worldwide, whereas race 3 has a much more limited geographical range (Martyn 2014, 2017). Race 3, originally identified in Maryland, was reported recently from Florida and Georgia (Amaradasa et al. 2018; Petkar et al. 2019; Zhou et al. 2010). Currently, race 2 is the most important race within the United States and other watermelon-producing countries because it is widely distributed, and no watermelon cultivars have resistance to race 2 (Martyn 2017). Although quantitative trait loci (QTL) that contain genes conferring resistance to race 2 have been identified in citron (*C. amarus*), watermelon cultivars that possess this resistance are not yet available (Branham et al. 2017; Wechter et al. 2012). Race 2 is present in South Carolina, based on identification of a limited number of isolates recovered from wilted watermelon plants from commercial farms submitted for diagnosis. Of 16 isolates, 15 and one were race 2 and race 1, respectively (Keinath and DuBose 2009).

Lack of reproducibility among repeated race tests has been observed, as discussed by Bruton et al. (2010). To address this variation, Zhou and Everts (2003) calculated mean wilt incidence across all cultivars tested, rather than relying on wilt incidence of a single cultivar to distinguish race 0 from race 1 and race 1 from race 2. Isolates with a mean wilt incidence <33% were designated as race 0, a mean wilt incidence 33 to 66% as race 1, and a mean wilt incidence >66% as race 2. In general, race 1 isolates caused ≤50% wilt incidence on Calhoun Gray, Dixielee, and Allsweet, whereas race 2 isolates caused wilt incidence >50% on these three cultivars, based on previous work by Hopkins et al. (1992). Isolates were considered nonpathogenic if they caused <15% wilt incidence on Sugar Baby, a cultivar susceptible to all races.

Because of the time required to identify races with pathogenicity tests, molecular methods have been suggested as a quicker alternative. Lin et al. (2010) designed primers based on a randomly amplified DNA fragment. Their Fon-1/Fon-2 primer set amplified a 174-base pair (bp) fragment from 13 FON isolates, including one isolate from South Carolina, but did not amplify DNA extracted from 12 other *F. oxysporum* formae speciales or five other *Fusarium* spp. Petkar et al. (2019) used these primers to identify FON isolates before conducting pathogenicity tests on their collection of isolates. FON race 0 and race 1 isolates recently were reported to possess copies

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of secreted-in-xylem (SIX) elicitor number 6, but this protein was absent in race 2 isolates (Niu et al. 2016). The FonSIX6 primers may be useful to identify races or to separate race 2 isolates from isolates of races 0 and 1. Niu et al. (2016) tested only seven isolates with their primers, so many more isolates need to be tested before the utility of these primers can be assessed.

Knowing which race is present in a field or on a farm allows growers to choose cultivars with partial resistance to *Fusarium* wilt. Diploid and triploid watermelon cultivars with resistance to races 0 and 1 are available commercially and used widely in the southern United States (Kemle et al. 2020). Triploid cultivars with resistance to race 1 also reduce the incidence of *Fusarium* wilt in fields with mixed populations of race 1 and race 2 (Keinath et al. 2010, 2019).

The objectives of this study were to i) determine the distribution and frequency of *F. oxysporum* f. sp. *niveum* races, particularly race 2, in the major watermelon-growing areas of South Carolina; ii) compare rating criteria for symptoms on cultigens with resistance to race 2; and iii) evaluate the specificity of two previously published primer sets on the collection of isolates from South Carolina (Lin et al. 2010; Niu et al. 2016).

Materials and Methods

Sources of FON isolates. *Research field.* In 2005, *Fusarium* wilt symptoms appeared in a research field at the Coastal Research and Education Center (CREC) in Charleston, SC (Keinath et al. 2006). Symptoms had not appeared on previous watermelon crops in this field. Symptomatic plants were collected for isolation of *Fusarium* spp.

Plants for diagnosis-Fields. Six samples obtained between 1999 and 2010 originated from wilted plants sent to CREC for diagnosis or confirmation of *Fusarium* wilt occurring in commercial fields of watermelon. Clemson University Cooperative Extension agents or growers selected these plants. The sample collected in Bamberg County in 2010 came from a spot in a field with a high incidence of symptomatic plants. One sample (Colleton County, 2008) originated from wilted plants selected by the first author on a visit to an affected field to confirm *Fusarium* wilt after a report of a disease outbreak.

Plants for diagnosis-Greenhouses. Outbreaks of *Fusarium* wilt on greenhouse-grown watermelon occurred in 2006 and 2018 in the same greenhouse in South Carolina. In 2006, pollenizer watermelon *C. amarus* cv. SP-1 was symptomatic. In 2018, four triploid watermelon cultivars, Fascination, Captivation, Road Trip, and Sweet Gem and pollenizer watermelon *C. amarus* cv. SP-6 were affected. In both cases, cotyledons of diseased seedlings were necrotic, while hypocotyls remained green. On most plants, the first true leaf had not yet emerged.

Commercial fields sampled. In 2008, diseased plants were collected from research plots in a 0.6-ha portion of a field in Hampton County that was part of an on-farm experiment (Keinath and Hassell 2009).

Between 2011 and 2013, six commercial watermelon fields were sampled to collect plants with symptoms of *Fusarium* wilt (Table 1). Collectors walked a diagonal path from corner to corner across each field. At approximately 3 m intervals, if any plants showed wilting of lower leaves, one plant was dug, placed in a plastic

Table 1. Distribution of races of *Fusarium oxysporum* f. sp. *niveum* on watermelon and pollenizer watermelon grown in research fields, commercial fields, and greenhouses in South Carolina and the percentage of isolates within each collection that matched the expected reaction with two PCR primer sets for the pathogen

					Correctly identified (%)												
					NP ^r			Race 0			Race 1			Race 2			
County	Year	Grower	Cultivar(s)	Resistance	N ^s	Fon ^t	SIX ^u	N	Fon	SIX	N	Fon	SIX	N	Fon	SIX	Total
Colleton	1999	1 ^v	Stars & Stripes	Susceptible	0	na ^w	na	0	na	na	0	na	na	2	nd ^x	nd	2
Charleston	2005	RF ^y	Various triploid	Susceptible	2	100	nd	0	na	na	13	100	8	22	100	100	37
Colleton	2005	1 ^v	StarGazer, Sugar Heart	Resistant, susceptible	0	na	na	0	na	na	0	na	na	2	100	0	2
Colleton	2005	1 ^v	StarGazer	Resistant	0	na	na	0	na	na	0	na	na	1	0	100	1
Colleton	2005	1 ^v	Sugar Heart	Susceptible	0	na	na	0	na	na	1	100	0	3	100	0	4
Barnwell	2006	GH ^z	SP-1	Susceptible	0	na	na	0	na	na	11	100	100	0	na	na	11
Bamberg	2008	Unknown ^v	Top Gun	Resistant	0	na	na	0	na	na	0	na	na	2	100	0	2
Colleton	2008	7 ^v	Tri-X 313, Palomar	Susceptible	0	na	na	0	na	na	0	na	na	5	100	20	5
Hampton	2008	2	SS7187	Susceptible	2	100	nd	0	na	na	0	na	na	17	7	100	19
Bamberg	2010	3 ^v	Palomar	Susceptible	1	100	100	1	0	0	1	100	100	9	100	0	12
Barnwell	2011	4	Revolution	Resistant	9	89	nd	0	na	na	0	na	na	0	na	na	9
Barnwell	2011	5	Revolution	Resistant	5	100	100	1	0	0	1	100	0	2	0	100	9
Colleton	2011	1	Liberty, Wrigley	Susceptible	1	100	100	0	na	na	3	67	67	15	80	33	19
Hampton	2012	2	Yellow Buttercup	Susceptible	0	na	na	1	100	100	2	100	100	7	57	50	10
Barnwell	2013	6	Bold Ruler	Susceptible	2	100	nd	0	na	na	12	100	67	10	100	100	24
Barnwell	2013	5	Revolution	Resistant	1	100	100	0	na	na	2	0	0	18	39	94	21
Barnwell	2018	GH ^z	Various triploid	Various	0	na	na	0	na	na	0	na	na	10	0	100	10
Total number of isolates					23	na	na	3	na	na	46	na	na	125	na	na	197
Percentage of all isolates					12	na	na	2	na	na	23	na	na	63	na	na	100
Percentage of pathogenic isolates					na	na	na	2	na	na	26	na	na	72	na	na	100
Percentage of isolates correctly reacting with primers					na	96	100	na	33	33	na	93	54	na	63	72	na

^r Races were identified based on greenhouse pathogenicity tests with three differential cultivars. Nonpathogenic isolates had a mean disease incidence $\leq 15\%$ (Zhou and Everts 2003).

^s Number of isolates identified as the given race.

^t Percentage of pathogenic isolates yielding or percentage of nonpathogenic isolates not yielding a 174-bp DNA fragment with the Fon-1/Fon-2 forma specialis-specific primers (Lin et al. 2010). Amplification is expected with all pathogenic isolates, regardless of race, but not with nonpathogenic isolates. Amplification was performed with 192 isolates.

^u Percentage of race 0 or 1 isolates or percentage of nonpathogenic or race 2 isolates not yielding a 430-bp DNA fragment with the FonSIX6 primers (Niu et al. 2016). Amplification is expected with race 0 or race 1 isolates but not with nonpathogenic or race 2 isolates. Amplification was performed with 173 isolates that included only four of the 23 nonpathogenic isolates.

^v Sample submitted to first author for diagnosis.

^w na = not applicable.

^x nd = not done.

^y RF = research field at the Clemson University Coastal Research and Education Center, Charleston, SC.

^z GH = commercial greenhouse in South Carolina.

bag, and stored in a cooler for transport to the laboratory. A maximum of 30 plants were collected per field.

Soil isolates. Soil samples were collected with a soil probe to 30 cm depth next to symptomatic plants on a commercial watermelon farm in Hampton County in 2008 (Keinath and Hassell 2009).

Recovery of FON isolates from plants and soil. Komada's medium, which is semiselective for *F. oxysporum*, was used for all isolations (Komada 1975). Small pieces (ca. 3 × 3 mm) that included discolored vascular tissue were cut from the base of the main stem or vine. Tissue pieces were surface disinfested in 0.5% sodium hypochlorite for 1 min, rinsed in sterile deionized water, and placed onto Komada's medium. Plates were held under cool-white fluorescent lights at 22 to 24°C for 5 to 7 days. Isolates resembling *F. oxysporum* with white to pale orange, fluffy aerial mycelium and tan to purple-brown colony reverse color were selected (Komada 1975). Soil (10 g) was suspended in 90 ml water, and dilutions were spread onto plates of Komada's medium. Isolates resembling *F. oxysporum* were selected from dilution plates as described above. All isolates were subcultured by spreading a microconidial suspension on water agar and selecting a single germinated conidium. Isolates were stored on dried filter paper at 4°C.

Pathogenicity tests. Pathogenicity tests to identify races were done in 1999 and between 2005 and 2018. Suspensions of microconidia for each *F. oxysporum* isolate were prepared in potato dextrose broth as described previously (Keinath and Hassell 2014a). Black Diamond was used as the cultivar susceptible to all races in tests done prior to 2008, and Sugar Baby was used in all subsequent tests. Charleston Gray was used as the cultivar with resistance to race 0 in most tests, except that Crimson Sweet was used in tests done in 2008 and 2009. Allsweet was used as the cultivar with resistance to races 0 and 1 in all tests. Watermelon was seeded in Fafard 3B potting mix (Sun Gro Horticulture, Agawam, MA) in 128-cell flats and grown in a greenhouse for ca. 14 days. Seedlings were inoculated when the first true leaf was fully expanded. Seedlings were removed from cells, and the roots were rinsed gently to remove adhering potting soil. Roots were dipped for 10 s in a suspension of 10⁶ microconidia/ml and transplanted into 10-cm plastic pots filled with Fafard 3B potting mix. For each cultivar-isolate combination, 20 plants were inoculated, and four plants were placed in each of five replicate pots. Twenty plants of each differential cultivar were dipped in water and planted as described to serve as the noninoculated control treatment. Two and three weeks after inoculation, the number of plants with no symptoms, wilt symptoms, and the number of dead plants were recorded. Mean percentage of wilted and dead plants was calculated for each cultivar and across all three cultivars (Zhou and Everts 2003). One or two isolates previously characterized as race 2 in 1999 (isolates 997 and 998) were included in the six tests done in 2005 and 2006. Two standard isolates previously characterized as race 1 (isolate B05-07) and race 2 (B05-30C1) were included in the remaining tests done starting in December 2006 (Keinath and Hassell 2014a; Wechter et al. 2012). A total of 50 tests were done with a median of 10 isolates per test (range 6 to 17). All isolates were tested twice, and a few isolates were tested three times (Zhou and Everts 2003). The greenhouse temperature was set at 24/21°C day/night, and supplemental lighting was not used. In tests done between 2005 and 2011, *F. oxysporum* was reisolated on Komada's medium from two living Allsweet plants from each of two pots (replications) inoculated with each isolate to confirm its presence in diseased plants (Keinath and Hassell 2014a; Komada 1975).

Soil isolates of FON collected from a commercial farm in 2008 were tested in a quick pathogenicity test on Sugar Baby seeds. Procedures were followed as described by Zhou and Everts (2003) except that two replications were used per test, and the tests were repeated. Race 1 isolate B05-07, race 2 isolate B05-30C1, and mineral salts solution were included in all tests as control treatments. Isolates that caused >15% mean disease incidence were inoculated onto Sugar Baby seedlings with the root-dip method described above. Race 1 isolate B05-07 and water were included as control treatments. Isolates that caused >15% disease on Sugar Baby seedlings were inoculated onto Sugar Baby, Charleston Gray, and Allsweet as

described above to determine race phenotype. Race tests were done twice. Race 1 isolate B05-07, race 2 isolate A08-44, and water were included in all tests as control treatments. *F. oxysporum* was reisolated from two Allsweet plants from each of two pots (replications) inoculated with each isolate to confirm its presence in diseased plants (Keinath and Hassell 2014a).

After pathogenicity tests were completed, five highly virulent race 2 isolates from South Carolina commercial fields were tested in an additional experiment on another set of cultivars to determine if any of them could be race 3 (Zhou et al. 2010). Each isolate selected caused a mean of >90% wilt incidence in the previous race tests. The experiment was done three times on 16 March 2018, 20 November 2018, and 23 January 2019, and each repetition is referred to as a trial. Three cultivars of *C. amarus* were used: 296341-FR (provided by W. P. Wechter, USDA), Carolina Strongback (provided by W. P. Wechter, USDA), and SP-6 (Syngenta Seeds, Woodland, CA). Seed of 296341-FR was limited, so it could only be included in trial one; SP-6 was substituted in trials two and three. All *C. amarus* cultivars are resistant to FON races 0, 1, and 2. *C. lanatus* Allsweet, resistant to FON races 0 and 1, served as a race 2-susceptible control treatment in all trials (Wechter et al. 2016; Zhou and Everts 2003). Two standard isolates previously characterized as race 1 (B05-07) and race 2 (B05-30C1) were included in each trial.

Seedlings, 12 to 20 per cultivar per test, were inoculated by dipping roots in microconidial suspensions or water as described above. Two and three weeks after inoculation, the number of symptomatic plants was recorded as described above. An additional category of plants with chlorotic or necrotic cotyledons and first or first and second true leaves was added to the ratings to account for symptoms that appeared on 296341-FR, Carolina Strongback, or SP-6 with some isolates. Pieces from the lower stem of selected cultivars were cultured as described above to isolate *F. oxysporum*. In trial one, plants of PI 296341-FR and Carolina Strongback from all four replications were cultured, along with Allsweet inoculated with race 1. In trial three, plants of SP-6 from two replications were cultured.

Data were analyzed with SAS PROC GLIMMIX (SAS, Inc., Cary, NC). Counts of diseased and dead plants were summed from all replicate pots within a trial and used to calculate wilt incidence for each cultivar, which was transformed by calculating the arcsine of the square root before analysis. Cultivar, isolate, and trial were considered fixed effects, and replication was considered a random effect. Cultivar-isolate means were compared with Fisher's protected least-significant difference test evaluated at $P = 0.05$.

Reactions with FON primers. *Fusarium* cultures stored on dried filter paper were removed from storage at 4°C and grown on quarter-strength potato dextrose agar for 5 days at 22°C. Five 0.5-cm² sections of each isolate were transferred into 50 ml potato dextrose broth and shaken at 150 RPM at 22°C for 7 days. The culture liquid was removed by filtering through sterile cheese cloth. Hyphae (100 mg) were transferred to a 1.5-ml tube. DNA was extracted using a DNeasy plant mini kit (Qiagen, Germantown, MD), and genomic DNA was quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA).

PCR amplifications were performed using a Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA). Reaction mixtures consisted of 1 µl (10 ng/µl) of purified genomic DNA, 6.25 µl of 2× master mix of Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies), 0.1875 µl of a 1:500 dilution of 1 mM reference dye, and 0.5 µl of 10 µmol/liter forward and reverse primers for a total reaction volume of 12.5 µl. Amplification conditions were 95°C for 10 min and 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min and extension at 70°C for 1 min, followed by 95°C for 1 min, 55°C for 30 s, and 95°C for 30 s for a dissociation curve. Two different primer sets were used in separate reactions. The FON forma specialis-specific primers designated Fon-1/Fon-2 developed by Lin et al. (2010) were used on 190 isolates, and primers developed to detect the FonSIX6 gene by Niu et al. (2016) were used with 184 isolates (Table 1). Results were analyzed with MxPro QPCR Software version 4.10 (Agilent Technologies). A nontemplate control of water and race 2 isolate B05-30C1 were used as negative and

positive controls, respectively, in every set of reactions. When the FonSIX6 primers were used, race 1 isolate B05-07 also was used as a positive control. An initial reaction with *F. oxysporum* f. sp. *melonis* isolate 26406 (which originated from Illinois in 2013) was used to demonstrate no false positive reactions resulted with either primer set (data not shown). Watermelon actin primers R2 (tcacacttcgatgaggtt) and F1 (gaaattgtccgkacatgaa) were added to DNA of isolates that did not amplify or had high Ct values ($C_t > 28$) with the Lin et al. (2010) or Niu et al. (2016) primers, and the protocol listed above was run to confirm that the DNA was amplifiable.

Results

Race identification with pathogenicity tests. *Plant isolates.* Twenty-three (12%) of the 197 *F. oxysporum* isolates recovered from plants were not pathogenic on watermelon, based on the criterion of mean disease incidence $\leq 15\%$ on Sugar Baby used by Zhou and Everts (2003). All isolates also had mean incidence $\leq 15\%$ across all three cultivars calculated for both trials. Two isolates caused no disease on any plants in either of two trials. Twelve isolates had mean disease incidence $< 33\%$ in one trial and no disease on any cultivar in the other trial. The other nine isolates classified as nonpathogenic had low disease incidence in both trials.

Pathogenic isolates accounted for 88% of the total number of plant isolates. Of the 174 pathogenic isolates, 2% (three isolates) were race 0, 26% (46 isolates) were race 1, and 72% (125 isolates) were race 2. All three isolates classified as race 0 had a mean incidence $> 15\%$ on cv. Sugar Baby but an overall mean incidence $< 33\%$ across the three differential cultivars (Zhou and Everts 2003).

All 46 isolates classified as race 1 had mean incidence $< 50\%$ on Allsweet, the differential cultivar for determining whether an isolate is race 1 or race 2. Three race 1 isolates from commercial fields did not cause any disease on Allsweet, and two additional isolates each caused disease on only one of 40 plants. Seven of the 46 race 1 isolates had a mean incidence of 67 or 68% across all three differential cultivars, but because mean incidence on Allsweet was $\leq 40\%$, these isolates were classified as race 1, even though they marginally exceeded the upper threshold of 66% mean incidence used by Zhou and Everts (2003).

All 125 isolates classified as race 2 had a mean incidence $> 50\%$ on Allsweet. Ten isolates from commercial fields had a mean incidence $< 66\%$ on the three differential cultivars but were classified as race 2 based on incidence on Allsweet of $\geq 53\%$. The oldest isolates were collected from a commercial field in 1999. Race 2 isolates were recovered from symptomatic plants more frequently than nonpathogenic, race 0, and race 1 isolates (t tests, $P = 0.0005$, $P = 0.0001$, and $P = 0.0008$, respectively). Susceptibility of the cultivars from which isolates were recovered did not affect the percentage of isolates that were nonpathogenic or that were classified into the three races (t tests, $P \geq 0.06$). *F. oxysporum* was recovered from all inoculated Allsweet plants cultured on Komada's medium.

Field-grown plants were the source of 153 of the 174 pathogenic isolates. The distribution of races among field isolates was 2% race 0 (three isolates), 23% race 1 (35 isolates), and 75% race 2 (115 isolates). Seven isolate collections were obtained by sampling fields systematically as described previously. Five collections consisted of a mix of nonpathogenic and pathogenic isolates, one collection included only pathogenic isolates, and one collection consisted of only nonpathogenic isolates. Two, five, and six of the six collections with pathogenic isolates included one or more isolates classified as race 0, 1, and 2, respectively (Table 1). Seven collections of isolates originated from plants submitted for diagnosis. Twenty-four, two, one, and one of the 28 isolates in these collections were classified as race 2, 1, 0, and nonpathogenic, respectively. The proportion of isolates that were identified as race 2 was greater in the diagnosis collections (0.93 ± 0.04 [standard error]) than in collections obtained by sampling fields (0.55 ± 0.13) ($P = 0.029$). Proportions of isolates identified as nonpathogenic, race 0, and race 1 did not differ between diagnosis and systematically sampled collections ($P \geq 0.10$). Twenty-one isolates originated from greenhouse-grown seedlings.

All 11 of the 2006 isolates were race 1, and all 10 of the 2018 isolates were race 2 (Table 1).

The field in Barnwell County planted in 2013 to the susceptible cultivar Bold Ruler had been planted yearly to watermelon for approximately 25 years, according to the grower. Among the 24 pathogenic isolates collected from symptomatic plants in this field, 12 were race 1 and 10 were race 2 (Table 1).

Soil isolates. Of the 81 soil isolates tested in the quick pathogenicity test on Sugar Baby seeds, 44 isolates caused no symptoms in either test, 22 isolates caused symptoms on $> 15\%$ of the seedlings in both tests, and 14 isolates caused symptoms on $> 15\%$ of the seedlings in only one test. One additional isolate was tested once with 100% disease incidence. Of the 53 isolates further tested on Sugar Baby seedlings with a standard root-dip inoculation, disease incidence was $\geq 50\%$ for 17 isolates, while incidence was $\leq 10\%$ for the remaining 36 isolates, which were considered nonpathogenic. Disease incidence in the quick pathogenicity test was correlated with disease incidence in the Sugar Baby root-dip test (Pearson correlation coefficient 0.841, $P < 0.0001$). However, incidences in the two inoculation methods were not correlated when only the 17 pathogenic isolates were included (Pearson correlation coefficient 0.323, $P = 0.32$). The 17 pathogenic soil isolates included five isolates of race 1 (29%) and 12 isolates of race 2 (71%).

Testing for race 3. In the experiment to detect race 3 isolates, the effects of isolate, cultivar, and the isolate-by-cultivar interaction were significant ($P \leq 0.0001$) in all three trials. Disease incidence based on wilting and death of inoculated plants was $> 65\%$ on Allsweet, the race 2-susceptible cultivar, with all race 2 isolates. Incidence with all race 2 isolates was significantly greater on Allsweet than on non-inoculated control plants and on plants inoculated with the standard race 1 isolate B05-07 (Tables 2 and 3). Incidence with the race 1 isolate B05-07 was $< 4\%$ and did not differ from the noninoculated control on any cultivar in any trial. In trial one, *F. oxysporum* was recovered from 92% of inoculated PI 296341-FR plants, 96% of inoculated Carolina Strongback plants, and 83% of Allsweet plants inoculated with race 1. In trial three, *F. oxysporum* was recovered from 57% of inoculated SP-6 plants but not from noninoculated plants. *F. oxysporum* was not recovered from plants inoculated with isolate JLP-13 or the standard race 1 isolate.

In general, disease incidence with race 2 isolates, including the standard race 2 isolate B05-30C1, was significantly greater on Allsweet than on the three cultivars of *C. amarus* with resistance to race 2, except on Carolina Strongback inoculated with isolates F11-10 and JLP-13 in trial one and isolates A08-12 and NP-2 in trial two (Tables 2 and 3). In trial one, disease incidence for all isolates on PI 296341-FR was significantly lower or equally low ($\leq 6\%$) compared with incidence on Carolina Strongback (Table 2). In trial two, isolates A08-12, NP-20, JLP-13, and B05-30C1 had higher incidence on Carolina Strongback than on the noninoculated control, and isolates JLP-13 and BG-05 had significantly higher incidence on SP-6 than on the noninoculated control (Table 3). Isolates A08-12, NP-20, and B05-30C1 had higher incidence on Carolina Strongback than they did on SP-6, but incidence did not differ between Carolina Strongback and SP-6 with the other isolates. In trial three, only isolate JLP-13 had higher incidence on Carolina Strongback than on the noninoculated control; on SP-6, no isolates differed from the noninoculated control. There were no significant differences between Carolina Strongback and SP-6 with any isolate ($P = 0.05$) (Table 3).

Mean disease incidence based on wilting and death caused by race 2 isolates was greatest on Allsweet ($94.0 \pm 3.2\%$ [standard error]), less on Carolina Strongback ($30.4 \pm 8.4\%$), and least on PI 296341-FR ($4.6 \pm 3.2\%$) and SP-6 ($4.6 \pm 2.4\%$). Based on the disease incidence values used by Martyn and McLaughlin (1983) for designating cultivar resistance, Allsweet was susceptible to race 2, Carolina Strongback was moderately resistant to race 2, and PI 296341-FR and SP-6 were resistant to race 2.

In the experiment to detect race 3 isolates, some plants exhibited an additional symptom besides wilting of the entire plant or plant death. The cotyledons and the first true leaf of inoculated plants turned yellow or became necrotic by 3 weeks after inoculation.

Yellowing or necrosis of leaves appeared on plants inoculated with all isolates but rarely on the noninoculated control plants. This symptom was more frequent on PI 296341-FR, Carolina Strongback, and SP-6 than on Allsweet (Tables 2 and 3). When plants with symptoms on the lowest leaves were included in disease incidence, mean incidence increased by $5.2\% \pm 2.0$ (standard error) ($P < 0.02$) on Allsweet, $59.8\% \pm 11.9$ ($P = 0.0041$) on PI 296341-FR, $30.8\% \pm 3.4$ ($P \leq 0.0001$) on Carolina Strongback, and $11.7\% \pm 3.5$ ($P = 0.006$) on SP-6 compared with incidence based only on wilted or dead plants (Table 2,3). This increase was observed with five race 2 isolates and the standard race 1 isolate (t tests, $P \leq 0.03$), but the difference was slight for the standard race 2 isolate ($P = 0.07$).

In the analysis of variance for disease incidence based on wilting, death, and yellowing, there was no isolate-by-cultivar interaction ($P = 0.21$) and no effect of cultivar ($P = 0.11$) in trial one, but isolate means differed from each other ($P \leq 0.0001$). In trials two and three, all three sources of variation were highly significant ($P \leq 0.0001$). In

all three trials, incidence based on wilting and yellowing on Allsweet was greater for all race 2 isolates than for the race 1 isolate and the noninoculated control (Tables 2 and 3). In trial one, incidence on PI 296341-FR with all race 2 isolates was $>70\%$ and greater than the noninoculated control. In trials one and two, incidence based on wilting and yellowing was $\geq 50\%$ on all inoculated Carolina Strongback plants than on the noninoculated control. In trial three, incidence based on wilting, death, and yellowing was greater on Carolina Strongback inoculated with race 2 isolates NP-20, JLP-13, and A08-12 and race 1 isolate B05-07 than on the noninoculated control. On SP-6, only a few race 2 isolates had greater incidence than the inoculated control in trials two and three (Tables 2 and 3).

On Carolina Strongback, incidence was greater with the race 1 isolate B05-07 than in the noninoculated control in all three trials (Tables 2 and 3). In trial one, six race 2 isolates had greater incidence than B05-07 (Table 2). In trial two, three race 2 isolates had greater incidence than B05-07, and three race 2 isolates had similar

Table 2. Incidence of Fusarium wilt on plant introduction (PI) 296341-FR and two other cultivars of *Citrullus* spp. inoculated with isolates of *Fusarium oxysporum* f. sp. *niveum* in the greenhouse^u

Isolate	Race	Plants wilted or dead (%)			Plants wilted, dead, or with yellowed/necrotic lower leaves (%)		
		Allsweet ^v	PI 296341-FR	Carolina Strongback	Allsweet	PI 296341-FR	Carolina Strongback
F11-10	2	100.0 a ^w	19.8 de	85.4 ab	100.0 a	90.8 abc	100.0 a
NP-20	2	97.7 a	0.0 f	2.3 ef	100.0 a	97.7 ab	90.8 ab
JLP-13	2	100.0 a	5.6 ef	66.7 bc	100.0 a	100.0 a	97.7 ab
A08-12	2	97.7 a	0.0 f	27.0 cde	97.7 ab	73.0 bc	100.0 a
BG-05	2	65.5 bc	0.0 f	5.6 ef	86.1 abc	90.8 bc	100.0 a
B05-30C1 ^x	2	100.0 a	2.3 ef	41.5 cd	100.0 a	100.0 a	100.0 a
B05-07 ^y	1	2.3 ef	ND	0.0 f	9.2 d	ND	58.5 c
NIC ^z	—	0.0 f	0.0 f	0.0 f	2.3 d	0.0 d	2.3 d

^u Least-squares mean of incidence. Incidence was determined by counting the number of wilted or dead plants and by counting the number of plants that were dead, wilted, or had yellow or necrotic lower leaves.

^v Cultivar Allsweet is *C. lanatus* resistant to races 0 and 1; the other two cultivars are *C. amarus* resistant to races 0, 1, and 2.

^w Means by symptom type with the same letters in columns (cultivar comparisons for each isolate) or rows (isolate comparisons for each cultivar) do not differ, Fisher's protected least-significant difference test, $P = 0.05$.

^x Standard race 2 isolate included as a control.

^y Standard race 1 isolate included as a control.

^z Noninoculated control treated with water instead of a microconidial suspension.

Table 3. Incidence of Fusarium wilt on three cultivars of *Citrullus* spp. inoculated with isolates of *Fusarium oxysporum* f. sp. *niveum* in the greenhouse^u

Trial	Isolate	Race	Plants wilted or dead (%)			Plants wilted, dead, or with yellowed/necrotic lower leaves (%)		
			Allsweet ^v	Carolina Strongback	SP-6	Allsweet	Carolina Strongback	SP-6
2	F11-10	2	100.0 a ^w	6.7 cde	1.7 de	100.0 a	56.5 b	10.3 cd
2	NP-20	2	100.0 a	96.2 a	1.7 de	100.0 a	100.0 a	10.3 cd
2	JLP-13	2	100.0 a	25.0 c	25.0 c	100.0 a	50.0 b	25.0 bc
2	A08-12	2	100.0 a	96.2 a	0.0 e	100.0 a	100.0 a	0.0 d
2	BG-05	2	100.0 a	3.8 cde	19.6 cd	100.0 a	56.5 b	25.0 bc
2	B05-30C1 ^x	2	100.0 a	69.1 b	3.8 cde	100.0 a	98.3 a	19.6 bc
2	B05-07 ^y	1	3.8 cde	1.7 de	0.0 e	10.3 cd	50.0 b	0.0 d
2	NIC ^z	—	0.0 e	0.0 e	0.0 e	0.0 d	3.8 cd	0.0 d
3	F11-10	2	98.9 a	1.1 cd	0.0 d	100.0 a	9.6 efgh	34.5 def
3	NP-20	2	93.3 a	6.7 cd	1.1 cd	97.6 ab	75.0 bc	12.8 efgh
3	JLP-13	2	97.6 a	12.8 c	1.1 cd	100.0 a	39.6 cde	50.0 cd
3	A08-12	2	95.7 a	1.1 cd	0.0 d	98.9 a	25.0 defg	1.1 h
3	BG-05	2	50.0 b	0.0 d	1.1 cd	93.3 ab	2.4 gh	6.7 fgh
3	B05-30C1 ^x	2	95.7 a	0.0 d	0.0 d	100.0 a	0.0 h	0.0 h
3	B05-07 ^y	1	1.1 cd	0.0 d	0.0 d	4.3 gh	25.0 defg	1.1 h
3	NIC ^z	—	0.0 d	0.0 d	0.0 d	0.0 h	1.1 h	0.0 h

^u Least-squares mean of incidence. Incidence was determined by counting the number of wilted or dead plants and by counting the number of plants that were dead, wilted, or had yellow or necrotic lower leaves.

^v Cultivar Allsweet is *C. lanatus* resistant to races 0 and 1; the other two cultivars are *C. amarus* resistant to races 0, 1, and 2.

^w Means by symptom type with the same letters in columns (cultivar comparisons for each isolate) or rows (isolate comparisons for each cultivar) do not differ, Fisher's protected least-significant difference test, $P = 0.05$.

^x Standard race 2 isolate included as a control.

^y Standard race 1 isolate included as a control.

^z Noninoculated control treated with water instead of a microconidial suspension.

incidence. In trial three, only one race 2 isolate had greater incidence, while four race 2 isolates had similar incidence (Table 3). On SP-6, incidence with B05-07 was very low and did not differ from the non-inoculated control (Table 3).

In trial one, incidence based on wilting, death, and yellowing for six race 2 isolates did not differ between Allsweet, a cultivar susceptible to race 2, and PI 296341-FR and Carolina Strongback, cultivars resistant to race 2. In trial two, incidence based on all symptoms was greater on Allsweet than on Carolina Strongback for three race 2 isolates and did not differ for the other three race 2 isolates. In trial three, incidence did not differ between Allsweet and Carolina Strongback except for race 2 isolate NP-20. Incidence was greater on Allsweet than on SP-6 for all race 2 isolates (Table 3).

Mean disease incidence based on wilting, death, and yellowing caused by race 2 isolates was greatest on Allsweet ($98.5 \pm 0.8\%$) and PI 296341-FR ($92.0 \pm 4.2\%$), less on Carolina Strongback ($66.7 \pm 8.9\%$), and least on SP-6 ($16.3 \pm 4.4\%$). Based on the disease incidence values used by Martyn and McLaughlin (1983), Allsweet and PI 296341-FR appeared susceptible, Carolina Strongback was slightly resistant, and SP-6 was resistant to race 2.

FON primers. *Fon-1/Fon-2 primers.* The Fon-1/Fon-2 FON forma specialis-specific primers developed by Lin et al. (2010) were used to amplify DNA extracted from 192 isolates of *F. oxysporum*. A positive qPCR reaction, i.e., a C_T value <30 , resulted with 120 (63%) of the isolates (Tables 1 and 4). The actin gene amplified in 50 DNA samples that did not yield a product with the Fon-1/Fon-2 primers, indicating the DNA was of amplifiable quality. No actin fragment was detected in two other samples, which were excluded from the datasets for both primers. The Fon-1/Fon-2 primers did not amplify DNA from 22 of the 23 nonpathogenic isolates or two of the three race 0 isolates. One nonpathogenic isolate collected from Barnwell County in 2011 and one race 0 isolate collected from Hampton County in 2012 yielded a product with these primers. Successful amplification was greater with race 1 than with race 2 isolates: 93% of race 1 isolates and 63% of race 2 isolates tested (Table 1). Twenty-six of 27 race 2 isolates from four collections did not react with the Fon-1/Fon-2 primers (Table 1). A single isolate from the 15 race 2 isolates tested from the 2008 Hampton County field yielded a product. Between 50 and 96% of the pathogenic isolates in the 10 other collections reacted with the primers. Overall, reactions with the Fon-1/Fon-2 primers matched pathogenicity identification 74% of the time.

FonSIX6 primers. Primers based on the FonSIX6 elicitor gene sequence described by Niu et al. (2016) were used to amplify DNA from 173 isolates. A positive qPCR reaction, i.e., a C_T value <30 , resulted with 60 isolates, and a C_T value >30 or no amplification resulted with the remaining 113 isolates. The amplified fragment, however, was 430 bp long for all isolates, not 739 bp as reported by Niu et al. (2016). A positive reaction with race 0 or race 1 isolates or no reaction with race 2 and nonpathogenic isolates was considered a match, i.e., positive identification of race. Reactions with the FonSIX6 primers matched race or pathogenicity identification 67% of

the time (Table 4). Isolates belonging to race 0, 1, and 2 were identified 33, 54, and 72% of the time. There was a 100% match with nonpathogenic isolates, although only four isolates were tested.

Results with the two primer sets agreed with each other for only 41% of the isolates (Table 4). Agreement was defined as the Fon-1/Fon-2 primers yielding a product for isolates identified as race 0, 1, or 2 and no amplification with DNA from nonpathogenic isolates, and the FonSIX6 primers yielding a product for race 0 or race 1 isolates and no amplification for all other isolates. Agreement was greatest for nonpathogenic isolates (75%), average for race 1 isolates (54%), and poor for race 0 (33%) and race 2 isolates (35%). Both primer sets yielded false positive results compared with the other primer set. The Fon-1/Fon-2 and FonSIX6 primers gave incorrect results for 26% and 30% of the isolates, respectively, when the other primer set matched the race identification correctly. False positive reactions with the Fon-1/Fon-2 were greatest for race 2 isolates, while both race 1 and race 2 isolates gave false positive reactions with the FonSIX6 primers (Table 4).

Discussion

The majority (72%) of FON isolates collected in South Carolina were race 2. The oldest isolates were collected from a commercial field in 1999. In 2000 in Maryland and Delaware, 22, 57, and 21% of the isolates recovered from symptomatic watermelon plants in commercial fields were race 2, race 1, and race 0, respectively, while 76% of the isolates from a research field in Maryland were race 2. Zhou and Everts (2003) explained the greater proportion of race 2 isolates in the research field was due to frequent planting of race 1-resistant cultivars, based on previous studies by Hopkins et al. (1992), who found that the proportion of race 1 and 2 isolates increases with cultivation of race 0- and race 1-resistant cultivars, respectively. The selective effect of cultivar resistance on the FON population could explain the current race distribution in South Carolina, where 2, 23, and 75% of field isolates were race 0, 1, and 2, respectively. Of six collections of field isolates obtained between 2011 and 2013, half originated from susceptible cultivars and half from cultivars resistant to races 0 and 1, while only one-quarter of collections made prior to 2011 originated from race 1-resistant cultivars (Table 1). In contrast to results from South Carolina, Petkar et al. (2019) identified only 39% of 59 isolates from Georgia and Florida as race 2 and no isolates as race 1. The proportion of race 0 isolates, however, was similarly low (5%), compared with the proportion in South Carolina.

Based on development of wilt symptoms or death of inoculated plants, five highly virulent isolates of FON obtained from diseased watermelons in five commercial fields in South Carolina were race 2, not race 3 (Tables 2 and 3). Disease incidence for all isolates was $<20\%$ on PI 296341-FR, substantially less than incidence on this cultigen reported for race 3 isolates from Maryland (78 to 100%) and Florida (100%) (Amaradasa et al. 2018; Zhou and Everts 2003). In addition, incidence for the five highly virulent race 2 isolates did

Table 4. Summary of primer reactions with isolates of *Fusarium oxysporum* f. sp. *niveum* identified to race in greenhouse pathogenicity tests

Race ^v	N ^w	FonSIX6 ^x match		FonSIX6 no match		% agree correct	% disagree wrong	% false positive Fon1/2	% false positive SIX6
		Fon1/2 match ^y	Fon1/2 no match	Fon1/2 match	Fon1/2 no match				
NP	4	3	1	na ^z	na	75	0	25	0
0	3	1	0	0	2	33	67	0	0
1	46	25	0	18	3	54	7	0	39
2	120	42	44	34	0	35	0	37	28
Total	173	71	45	52	5	41	3	26	30

^v Races were identified based on greenhouse pathogenicity tests with three differential cultivars. Nonpathogenic isolates had a mean disease incidence $\leq 15\%$ (Zhou and Everts 2003).

^w Number of isolates identified as the given race and tested with both primers.

^x FonSIX6 primers of Niu et al. (2016). Amplification is expected with race 0 or race 1 isolates but not with nonpathogenic or race 2 isolates.

^y Fon-1/Fon-2 forma specialis-specific primers of Lin et al. (2010). Amplification is expected with all pathogenic isolates, regardless of race, but not with nonpathogenic isolates.

^z na = not applicable.

not differ from incidence with the standard race 2 isolate, B05-30C1, on any of the four cultivars with only two exceptions that occurred on Carolina Strongback in only one trial each. (Petkar et al. [2019] confirmed this isolate is race 2.) In addition, incidence with the six race 2 isolates usually did not differ from incidence with the race 1 isolate on PI 296341-FR or SP-6. The readily available cultivar SP-6 may be a suitable cultivar to use in place of PI 296341-FR to identify isolates of race 3, since incidence based on wilting or death was $\leq 25\%$ with race 2 isolates. However, the reaction of SP-6 would have to be determined to additional isolates of race 2 and to isolates of race 3 before it could replace PI 296341-FR as a standard differential cultivar to detect race 3.

The Fon-1/Fon-2 FON forma specialis-specific primers of Lin et al. (2010) amplified DNA from only one nonpathogenic isolate, representing 4% of the isolates that were judged nonpathogenic, i.e., had low virulence or caused no wilt symptoms in repeated tests. Likewise, the FonSIX6 primers based on an elicitor gene sequence described by Niu et al. (2016) did not amplify DNA from any of the four nonpathogenic isolates tested. Although the sample of 23 nonpathogenic isolates was small, Fon-1/Fon-2 primers may be a useful alternative to pathogenicity tests with Sugar Baby to identify isolates of FON within a collection of *F. oxysporum* isolates. However, these primers may misidentify race 0 isolates as nonpathogenic isolates, since they did not react with two of the three race 0 isolates. Because the FonSIX6 primers amplify a fragment presumably present only in race 0 and 1 isolates, they would not be useful for identifying pathogenic isolates. The specificity of the Fon-1/Fon-2 primers of Lin et al. (2010) was better with race 1 isolates (94%) than with race 0 isolates (33%) or race 2 isolates (64%) from South Carolina. Petkar et al. (2019) screened isolates of FON with the Fon-1/Fon-2 primers prior to pathogenicity testing and genetic diversity analysis. They reported low genetic diversity of 99 isolates from Georgia and Florida. It is possible that prescreening isolates with the Fon-1/Fon-2 primers selected isolates that shared some common genetic regions and excluded isolates that would have increased the diversity of the populations. Amplification with the FonSIX6 primers of Niu et al. (2016) matched race identification for 66% of the South Carolina isolates, and specificity did not vary among the races. Neither set of primers, however, is reliable enough to replace pathogenicity testing to identify races. The primers recently designed by van Dam et al. (2018) based on candidate effector genes present in FON were not tested in this study. Their three sets of PCR primers correctly identified seven isolates of FON. Based on the reactions of the primers of Lin et al. (2010) and Niu et al. (2016) with the FON isolate collection used in this study, the primers by van Dam et al. (2018) also should be evaluated on a larger collection of FON isolates.

Variation between repeated pathogenicity tests has been reported previously (Amaradasa et al. 2018; Bruton et al. 2010; Martyn and Bruton 1989). Zhou and Everts (2003) devised mean wilt incidence across all differential cultivars inoculated to address variation between replicated tests and to facilitate designating a race for all isolates, instead of labeling isolates that yielded variable results as unknown, as Martyn and Bruton (1989) did. Races in the current study were designated using Zhou and Evert's thresholds, even though they inoculated six cultivars, whereas we inoculated three of the six cultivars they used. Mean incidence, calculated from all isolates tested by Zhou and Everts (2003) on the three cultivars we mainly used, Sugar Baby, Charleston Gray, and Allsweet, was 20, 64, and 83%, respectively, which fit into their criteria for designating races 0 (<33% mean incidence), 1 (>33% and <66% incidence), and 2 (>66%). The only conflict found in the current study was that 10 of 123 isolates of race 2 had mean wilt incidence <66% but wilt incidence >50% on Allsweet. We considered the traditional definition of race 2, isolates that cause >50% wilt incidence on cultivars resistant to race 1, as the more compelling criterion, so these isolates were designated race 2 (Hopkins et al. 1992).

In the severely infested field in Hampton County sampled in 2008, 29% of the soil isolates were race 1, while no race 1 isolates were recovered from symptomatic watermelon plants sampled at the same time (Keinath and Hassell 2009, 2014b). Likewise, only four isolates

were identified as race 0, race 1, or nonpathogenic among 28 isolates obtained from field-grown plants submitted for diagnosis between 1999 and 2010 (Table 1). FON race 2 has been described as more aggressive than race 1 (Martyn and Bruton 1989), which may explain why a greater proportion of plant isolates were race 2 than race 1. Greater aggressiveness of race 2 may also account for the high proportion of race 2 isolates recovered from diagnosis samples.

Variation in cultigen reaction to some FON isolates also was observed in this study with cultigens resistant to race 2, namely Carolina Strongback, SP-6, and PI 296341-FR. Some plants of all three cultigens, plus the few surviving plants of Allsweet, had yellow or necrotic symptoms on the cotyledons and first, or rarely, second true leaves, but no wilting or other symptoms on the rest of the plant. For all isolate-cultigen combinations, disease incidence was higher when this resistant reaction was included than when it was excluded from the ratings. Moreover, when all symptoms were rated, disease incidence on PI 296341-FR, the main source of resistance to FON, did not differ from disease incidence on Allsweet, a standard race 2-susceptible cultivar. In addition, incidence was greater on Carolina Strongback, a rootstock cultivar with resistance to race 2, than on Allsweet for some race 2 isolates. Although Carolina Strongback is resistant to race 1, race 1 isolate B05-07 caused higher incidence than the noninoculated control in all three trials when yellowing and necrosis of the lower leaves of Carolina Strongback were included in incidence assessments. Thus, necrosis on the lower leaves should not be included in the disease ratings assigned to PI 296341-FR and Carolina Strongback inoculated with FON. Amaradasa et al. (2018) describe complete wilting of PI 296341-FR with a race 3 isolate in four repeated experiments and 67% wilting with a race 2 isolate in one of four tests. Zhou et al. (2010) and Petkar et al. (2019) included yellowing and stunting in their assessment of symptoms caused by race 2 and race 3 isolates inoculated onto PI 296341-FR, which in our opinion could cause some isolates to be misidentified as race 3. Including yellowing and stunting in disease ratings of PI 296341-FR could be one reason the proportion of race 3 isolates (56%) identified by Petkar et al. (2019) was higher than proportions reported in other states. In Maryland, one isolate of race 3 was found among 63 isolates (1.6%) collected from commercial farms in 2000 (Zhou and Everts 2003; Zhou et al. 2010). In Florida, 10 of 26 FON isolates (35%) were race 3 (Amaradasa et al. 2018), based on complete wilting.

Because most of the *F. oxysporum* f. sp. *niveum* isolates from South Carolina were race 2, integrated management practices should be used until commercial cultivars with resistance to race 2 are available (Branham et al. 2017). These practices include cover cropping with hairy vetch, delaying transplanting until the soil temperature reaches 27°C or greater, choosing cultivars with tolerance or race 1 resistance that produce medium-sized fruit, and applications of prothioconazole at transplanting (Keinath et al. 2010, 2019; Miller et al. 2017; Sanders et al. 2012; Zhou and Everts 2007). Alternatively, grafting watermelon onto interspecific hybrid squash or bottle gourd provides a high level of protection against Fusarium wilt in naturally infested soil (Keinath and Hassell 2014a, b).

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