Suppression of Fusarium Wilt of Watermelon by Nonpathogenic *Fusarium oxysporum* and Other Microorganisms Recovered from a Disease-Suppressive Soil

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ABSTRACT

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Nearly 400 microorganism isolates, including bacteria, actinomycetes, and fungi, were collected from watermelon roots growing in soils suppressive and nonsuppressive to Fusarium wilt of watermelon. These isolates were screened for their ability to restore suppressiveness to microwave-treated suppressive soil and to reduce disease incidence in conducive field soil. Specific isolates of nonpathogenic *Fusarium oxysporum* from suppressive soil were the only organisms consistently effective in reducing disease (35 to 75% reduction) in both microwave-treated and natural field soils. Thus, we concluded that *F. oxysporum* was the primary antagonist responsible for suppression in this suppressive soil, although other organisms may contribute to suppressiveness. Selected isolates of *F. oxysporum* were effective in reducing disease when added to field soils at inoculum levels as low as 50 to 100 chlamydospores per g of soil, which was comparable to or below pathogen inoculum levels (100 to 200 CFU/g of soil). Root colonization data indicated that reduction of disease was not directly related to the ability of the antagonist to colonize roots extensively or to reduce colonization by the pathogen. Effective antagonists were not associated with specific vegetative compatibility groups, indicating antagonists represent diverse isolates. In split-root experiments, in which the antagonist and the pathogen were physically separated from each other, root colonization by selected isolates of *F. oxysporum* reduced disease incidence, verifying the mechanism of action as induced systemic resistance. Several isolates of *F. oxysporum* from this suppressive soil have potential for development as biocontrol agents.

Additional keywords: *Fusarium oxysporum* f. sp. *niveum*, *Citrullus lanatus*, rhizoplane, rhizosphere, soil microbiology.

Soils that naturally suppress Fusarium wilt of numerous crops, caused by pathogenic forma speciales of *Fusarium oxysporum* Schlectend.:Fr., occur in several regions of the world (1,6,37,43). In these soils, disease does not readily develop even though the pathogen and susceptible hosts are present. Among the most extensively studied examples of Fusarium wilt-suppressive soils are those of the Chateaurenard region of France (1,4,24,27) and the Salinas Valley of California (37,38,41,43). In these and other such soils, disease suppression is associated with inhibition of chlamydospore germination and reduced saprophytic growth of the pathogen compared with conducive soils (9,11,24,27,37,38,41). These soils have several characteristics in common, including their general physical characteristics (high pH, organic matter, and clay content) and a large diverse population of antagonistic bacteria and actinomycetes associated with these characteristics (1,6,37,38,43). In addition to the general suppression provided by the large bacterial biomass, the specific cause of disease suppression in these soils has been attributed primarily to the activity of nonpathogenic *F. oxysporum* and fluorescent pseudomonads (3,4,33,37–39,41,42). Mechanisms responsible for disease suppression by nonpathogenic *F. oxysporum* include saprophytic competition for nutrients (1,9,24,27), parasitic competition for infection sites (39), and induced systemic resistance (ISR) (19,28,31,32,40). Fluorescent pseudomonads control disease by successfully competing for iron and nutrients and through antibiosis by the production of antifungal compounds (11,37,38,41). Antagonists isolated from suppressive soils and developed as biocontrol agents have successfully reduced disease in a number of greenhouse and field trials (3,4,13,25,34).

Soil suppressiveness to Fusarium wilt of watermelon also has been induced by successive cultivation (monoculture) of a specific watermelon cultivar (17,21,22). The characteristics of this monoculture-induced suppressive soil are quite different from those of the naturally occurring wilt-suppressive soils (1,6,21,22,43). The induced suppression of Fusarium wilt occurs in a soil type (sandy, low organic matter, and low pH) that is normally highly conducive to disease and does not result in inhibition of chlamydospore germination or reduced saprophytic growth of the pathogen (22). Because of these unique aspects, this suppression may involve different organisms, mechanisms, or interactions that differ from other wilt-suppressive soils, and biological components from this soil may be effective under soil conditions normally more conducive to disease development.

Our previous work with this suppressive monoculture soil focused on the general ecological characteristics of the pathogen in relation to other indigenous groups of microorganisms in suppressive versus conducive soil and the effect of watermelon cultivation on these characteristics (21,22). The results suggested that specific antagonistic organisms, rather than general population levels of microorganisms, were responsible for suppressiveness. Therefore, in the current study, potential antagonists were isolated from suppressive soil and evaluated for their possible roles in disease suppression. Because previous work indicated that indigenous strains of *F. oxysporum* were likely involved in suppression, special emphasis was placed on the interaction of the pathogen with isolates of *F. oxysporum* not pathogenic to watermelon. Because the patho-

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gen is biologically active within the rhizosphere and must invade the root before disease can result, we focused on rhizosphere- and rhizoplane-colonizing organisms.

The objectives of this study were to isolate a wide variety of microorganisms potentially antagonistic to *F. oxysporum* from the roots of watermelon plants growing in suppressive and nonsuppressive soil, screen them for their ability to restore suppressiveness and reduce disease in microwave-treated and nontreated field soil, and determine the mechanism of action and which characteristics of successful antagonists contribute to their effectiveness.

MATERIALS AND METHODS

Soil infestation and assay of Fusarium wilt. The four soils used in this study to represent different suppressive and conducive conditions were described previously (22) and consisted of a wilt-suppressive soil that was developed through monoculture to the moderately resistant watermelon cultivar Crimson Sweet (CSS); a nonsuppressive soil that had undergone monoculture to the susceptible watermelon cultivar Florida Giant (FGM); a conducive fallow soil that had not been planted to watermelon in 8 years (LFC); and a suppressive soil that had been rendered conducive by microwave treatment for 90 s/kg of soil (700 W, 2,450 MHz) at a soil matric potential of –10 kPa (CSMW). All soils, which were collected from field plots at the Central Florida Research and Education Center, Leesburg, are of the Apopka Fine Sand soil series (pH 6.0 to 6.5, organic matter content < 1%, clay content < 3%) and differ only in their cropping history and resulting biology. Soils were collected in large buckets, sieved through a 0.2-cm screen, and stored in plastic bags for up to 4 months before use.

An orange mutant (OM) strain of the pathogen *F. oxysporum* f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans., which was comparable to the parental wild-type of a race 1 strain in growth, pathogenicity, and root colonization, was used to distinguish the pathogen from indigenous *F. oxysporum* in the field soils (22). Soils were infested with chlamydospore inoculum of the OM pathogen (200 to 400 CFU/g of soil) as described previously (22). Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) seeds were planted in pots of infested soil in the greenhouse. There were four to six plants in each of four to six replicate pots depending on the individual experiment. Plants were maintained at 20 to 30°C and were grown for 4 weeks. Fusarium wilt was assessed by visual inspection of the plants for wilt symptoms several times a week and verified periodically by plating surface-disinfested stem pieces on Komada's (18) selective medium for *F. oxysporum*. Wilt incidence was expressed as the percentage of diseased plants over the 4-week period.

Isolation of potential antagonists. Roots from Crimson Sweet watermelon plants grown in CSS and FGM soils microwave-treated for 0, 30, or 60 s/kg of soil at a matric potential of –10 J/kg were used for rhizosphere and rhizoplane organism-dilution platings as described previously (22). Bacterial isolates were obtained by dilution plating on nutrient agar and 1/10-strength tryptic soy agar plates. Actinomycetes were selected for on alkaline water agar at pH 10.5 (16). Pseudomonads were isolated on a selective King's medium B (KB) containing penicillin, cycloheximide, and novobiocin (36). Plates of KB medium were examined under ultraviolet light for colonies producing diffusible fluorescent pigments to identify fluorescent strains. Fungal organisms were isolated on potato dextrose agar (PDA) containing 1 ml of tergitol NP-10 and 50 mg of chlortetracycline per liter of medium. *F. oxysporum* was isolated from surface-disinfested roots as well as rhizosphere and rhizoplane dilutions with Komada's (18) medium. Four replicate plates were prepared for each treatment dilution and incubated at 26°C as described previously (22). Isolates to be tested as potential antagonists were selected randomly from the agar plates, such that representative organisms from all colony and morphology types present were collected in approximate proportion to their abundance on the plates. Organisms other than *F. oxysporum* and fluorescent pseudomonads were not identified but were assigned isolate numbers based on the treatment and medium from which they were isolated. An additional 40 isolates of *F. oxysporum* that had been collected from bulk CSS soil samples in a previous study (20) also were tested as antagonists. Among these were four isolates that were weakly pathogenic on watermelon (VCG 0081, race 1).

Screening isolates in microwave-treated and field soils. All collected isolates were screened individually for their ability to restore suppressiveness and reduce disease when added to microwave-treated suppressive soil infested with chlamydospore inoculum of the pathogen at 200 to 400 CFU/g of soil. This test was used as an initial screening stage to identify organisms with any antagonistic potential toward Fusarium wilt and to reduce the total number of organisms that would need to be screened in field-soil tests, because only isolates that indicated antagonistic activity in microwave-treated soil would be tested further.

Fungal isolates were grown for 5 days on PDA at 26°C, conidial suspensions of 1 to 2×10^6/ml were prepared in sterile deionized water, and 10 ml of each suspension was mixed into 1 kg of infested, microwave-treated suppressive soil. Bacterial isolates were grown on nutrient agar for 48 h, suspended in sterile water, and adjusted to an optical absorption of 0.5 (read at 600 nm), which produced cell counts in the range of 10^7 to 10^9/ml. A 10-ml aliquot of this suspension was mixed into 1 kg of soil. Final concentrations of the potential antagonists added to soil were 1 to 2×10^4 CFU/g of soil for *F. oxysporum* and most other fungi and 10^5 to 10^7 CFU/g of soil for most bacteria. Seeds of watermelon cultivar Crimson Sweet were planted (five replicate pots of five seeds per pot), and wilt was assessed over 4 weeks. Data were compared with pathogen-infested CSS and CSMW control soils for percent wilt, percent suppression (relative to pathogen-infested CSS soil), and percent reduction in disease (relative to pathogen-infested CSMW soil).

All isolates providing a significant reduction of disease in microwave-treated soil were tested for their ability to reduce disease in a conducive field soil (LFC) infested with chlamydospore inoculum of the pathogen at 200 to 400 CFU/g of soil. Isolates were cultured in the same way as for the microwave-treated–soil tests, except bacterial cell suspensions were washed twice in sterile water before being mixed into pathogen-infested soil. Selected isolates of *F. oxysporum* that suppressed disease in field soil with conidial inoculum also were tested with chlamydospore inoculum at 50 to 200 CFU/g of field soil. Chlamydospores of antagonists were produced as described previously for pathogen inoculum (22). All field-soil screening tests were conducted at least twice.

Characteristics of successful antagonists. Internal colonization of watermelon roots by the OM pathogen and other isolates of *F. oxysporum* was determined for selected treatments at the conclusion of the screening trials in both microwave-treated– and field-soil tests. Surface-disinfested root systems (0.5% sodium hypochlorite for 1 min) were embedded in molten Komada's medium according to methods described previously (22). Plates were incubated at 26°C, and colonies emerging from roots were counted 3 to 7 days later. Colonies of the OM pathogen, antagonist *F. oxysporum*, and other fungi were easily distinguished on the plates. Colonization was expressed as number of colonies per 100 cm of root. Root colonization by the pathogen and antagonist isolates of *F. oxysporum* was evaluated in relation to the ability of each isolate to effectively reduce disease.

Vegetative compatibility was evaluated among a total of 42 isolates of *F. oxysporum*, including isolates from CSS and FGM soil as well as those that were successful and unsuccessful in reducing disease. Nitrate-nonutilizing mutants of selected isolates were produced on a chlorate-amended medium and tested for vegetative compatibility as described previously (20). Pairings were made with tester isolates for established vegetative compatibility groups (VCGs) within *F. oxysporum* f. sp. *niveum* (VCG 0080, 0081, and 0082) as well as all combinations among the test isolates (20).

ISR. A split-root technique was employed to test ISR as a possible mechanism of suppression and to separate this response from competition at or on the roots. This enabled the physical separation of the pathogen from the antagonists and indigenous microorganisms in suppressive soil. Watermelon seeds were germinated in moist paper towels. Upon emergence of the developing root, the root tip was excised, and the root was allowed to grow for two to three more days. This resulted in a proliferation of rootlets near the point of excision. A number of rootlets were separated into two similar halves for transplanting to a dual-pot system consisting of two square plastic pots (6.5 cm wide × 9 cm high) taped together (Fig. 1). One pot of each pair contained CSMW, CSS, LFC, or CSMW soil with an antagonist added. The other pot of each treatment contained 100 g of CSMW soil with the OM pathogen added at 400 CFU/g of soil in the bottom half of the pot plus 150 g of CSMW soil without the pathogen in the top half of the pot. This setup enabled roots to grow 2 to 3 days through suppressive or antagonist-infested soil before being challenged through colonization by the pathogen in the adjoining pot. For the antagonist treatment, one half of the root was dipped in a conidial suspension (10^6 CFU/ml) of a nonpathogenic isolate of *F. oxysporum* before being transplanted into CSMW soil.

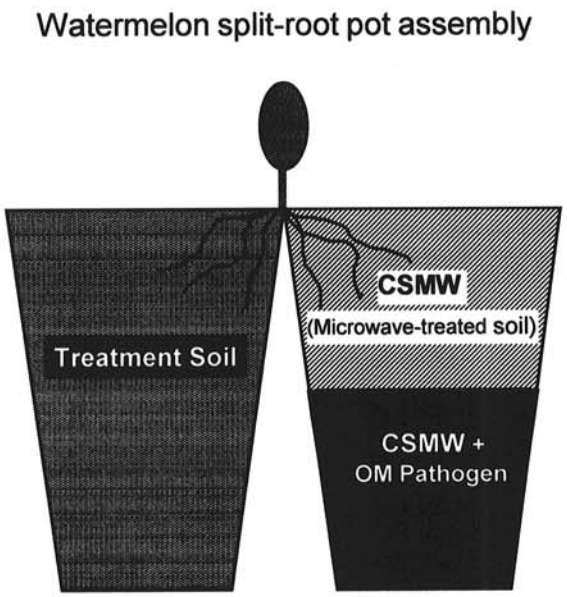

Fig. 1. Diagram of a watermelon plant in a split-root pot assembly (treatment soil = suppressive monoculture soil; conducive field soil; microwave-treated suppressive soil; or microwave-treated suppressive soil supplemented with an antagonistic isolate of *Fusarium oxysporum* not pathogenic on watermelon. CSMW = microwave-treated suppressive soil; CSMW + OM pathogen = microwave-treated soil with the orange mutant (OM) pathogen added at 400 chlamydospores per g of soil).

Experiments consisted of 16 to 24 split-root plants for each treatment (four to six replicates of four plants each). Plants were allowed to grow 5 weeks. Root systems and stem sections of wilted plants were surface-disinfested and plated on Komada's medium. All plants were plated at the conclusion of the experiment. This study was conducted twice.

Statistical analyses. Statistical analyses were conducted by the general linear models procedures of Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC). Experiments were designed as randomized complete blocks with factorial treatment structures and analyzed by standard analysis of variance with interactions. Significance was evaluated at $P < 0.05$ for all tests. Mean separation was accomplished by Duncan's multiple range test or orthogonal contrasts. All data expressed as percents were arcsine-transformed ($\sin^{-1}\sqrt{x}$) prior to analysis. Summary information for each antagonist isolate (including whether the isolate significantly reduced disease and the degree of disease reduction) was combined for all screening tests, and differences in organism group characteristics were determined by orthogonal class comparisons or chi-square tests.

RESULTS

Isolation and screening of potential antagonists. A total of 140 isolates of *F. oxysporum* and 255 isolates of miscellaneous bacteria, actinomycetes, and fungi were collected from the roots of Crimson Sweet watermelon plants grown in suppressive and nonsuppressive soil (Table 1). Most of the isolates, 110 *F. oxysporum* and 170 other microorganisms, were collected from suppressive soil. Isolates from nonsuppressive soil were collected primarily for comparative purposes. In addition to *F. oxysporum*, a total of 35 other fungal isolates, including other species of *Fusarium*, such as *F. solani* and *F. equisetii*, and species of *Trichoderma*, *Penicillium*, and *Aspergillus* were collected. The 220 bacterial isolates consisted of 40 fluorescent pseudomonads, 30 actinomycetes, and 150 unidentified bacterial isolates.

TABLE 1. Potential antagonistic organisms isolated from watermelon roots in Fusarium wilt-suppressive and -nonsuppressive soil and their ability to reduce disease in infested microwave-treated suppressive soil

Organism group[w]	Soil used[x]	Microorganism isolates[y]				
		Total number	Disease reduction		>50% Reduction (%)	Avg. reduction (%)
			Number	Percent		
F. oxysporum	CSS	110	68	62 a[z]	55 a	47.1 a
	FGM	30	9	30 b	27 b	25.3 b
Other fungi	CSS	25	4	16 b	12 b	17.9 bc
	FGM	10	1	10 b	0 b	4.6 c
Fluorescent pseudomonads	CSS	30	7	23 b	10 b	26.6 b
	FGM	10	2	20 b	0 b	25.6 b
Other bacteria	CSS	120	29	24 b	12 b	23.3 b
	FGM	30	7	23 b	3 b	18.5 bc
Actinomycetes	CSS	20	5	25 b	8 b	30.4 b
	FGM	10	3	30 b	10 b	29.5 b

[w] Komada's selective medium was used for isolating *F. oxysporum*; all other fungi were isolated on potato dextrose agar amended with tergitol and chlortetracycline; fluorescent pseudomonads were isolated on selective King's medium B; all other bacteria were isolated on nutrient agar and 1/10-strength tryptic soy agar; actinomycetes were selected for on alkaline water agar, pH 10.5.

[x] CSS = suppressive Crimson Sweet monoculture soil; FGM = nonsuppressive Florida Giant monoculture soil.

[y] Values represent the total number of isolates within each group tested, the number and percentage of isolates that resulted in a significant ($P < 0.05$) reduction of disease incidence (relative to pathogen-infested control soil), the percentage of isolates that reduced disease by more than 50%, and the average percent reduction in wilt incidence for all isolates within each group in microwave-treated soil-screening tests.

[z] Values within each column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Separate screening trials (15, each consisting of 20 to 30 individual isolate treatments) were required to screen all 395 isolates for their potential as antagonists to Fusarium wilt in CSMW soil. Disease incidence in the pathogen-infested microwave-treated control soil ranged from 55 to 100% wilt and averaged 81 ± 7% (standard error) wilt over all the individual screening tests. Disease in the pathogen-infested suppressive soil control ranged from 5 to 42% wilt, with an average of 28 ± 7% wilt over all tests. Disease levels in individual isolate treatments ranged from 5 to 100% wilt. To standardize the isolate effects and make comparisons over several screening tests with varying disease levels, isolate effectiveness was expressed as the percent reduction of disease relative to infested CSMW control soil in each test. Isolates capable of substantially restoring suppressiveness and significantly ($P < 0.05$) reducing disease in CSMW soil were considered effective antagonists in this soil. Isolates that reduced disease by 50% or more and maintained low levels of wilt comparable to the suppressive soil were considered highly effective antagonists.

Effective isolates were found within all general organism types and in both suppressive and nonsuppressive soils (Table 1). However, isolates of *F. oxysporum* collected from suppressive soil were significantly more effective than any other type of organism. Isolates of *F. oxysporum* from suppressive soil were responsible for a larger number and larger proportion of effective isolates (62%) than any other group, including isolates of *F. oxysporum* from nonsuppressive soil (30%). All other microorganism groups contained relatively few effective isolates; no significant differences were observed in the proportion of effective isolates between suppressive and nonsuppressive soils (10 to 30%). In addition, the majority of isolates of *F. oxysporum* were highly effective in reducing disease, whereas organisms from other groups tended to be only marginally effective in reducing disease. Overall, isolates of *F. oxysporum* from suppressive soil reduced disease an average of 47.1%, versus reductions of 4.6 to 30.4% for all other organism types tested (Table 1). Results from one typical screening trial are presented in Figure 2 and are representative of the overall results of numerous tests in which a high percentage of isolates of *F. oxysporum* and occasionally other organisms significantly reduced disease in microwave-treated soil.

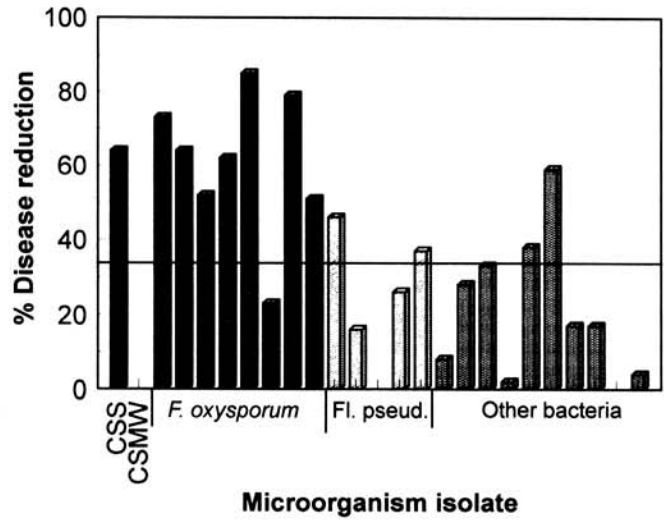

Fig. 2. Percent reduction of incidence of Fusarium wilt of watermelon by various potential antagonist isolates of *Fusarium oxysporum*, fluorescent pseudomonads, and other bacteria in a typical screening trial in microwave-treated soil (CSS = pathogen-infested suppressive soil control; CSMW = pathogen-infested microwave-treated soil control). Horizontal line indicates the level of significant reduction of disease (LSD, $P = 0.05$). All bars extending above this line represent isolates that significantly reduced disease. Average wilt in CSMW control soil was 80%.

A total of 60 isolates of *F. oxysporum* and 62 isolates of other organisms that were effective antagonists in microwave-treated soil were screened in conducive field soil. Disease incidence ranged from 55 to 96% wilt in pathogen-infested control soil and averaged 73 ± 4% wilt over the eight separate screening trials. There was a wide variation among isolates of *F. oxysporum* from suppressive soil in their ability to reduce disease (Fig. 3A). Of 51 isolates of *F. oxysporum* from suppressive soil tested, 20 (39.2%) significantly reduced disease in at least one field soil test. Of nine isolates from nonsuppressive soil, only one (11.1%) reduced disease in any field soil test. In general, bacteria, actinomycetes, and other fungal isolates were poor antagonists when tested individually in field soil. Of 62 other organisms tested (42 from CSS soil and 20 from FGM soil), only 4 bacterial isolates from CSS soil (9.5%) showed a reduction of wilt in any test (Fig. 3B). However, these bacterial isolates did not consistently reduce disease in repeated tests.

As a group, isolates of *F. oxysporum* were significantly ($P < 0.05$) more effective in reducing disease than all other organisms, with 35% of all *F. oxysporum* and only 7% of other organisms effectively reducing disease in field-soil tests. Additionally, isolates of *F. oxysporum* from CSS soil were significantly more effective ($P < 0.05$) in controlling disease than any other organism group, reducing disease by an average of 27.1% compared to reductions of 12.3, 15.7, and 10.0% for *F. oxysporum* from FGM soil and bacteria from CSS and FGM soils, respectively. All isolates from CSS soil, including *F. oxysporum*, bacteria, and other organisms,

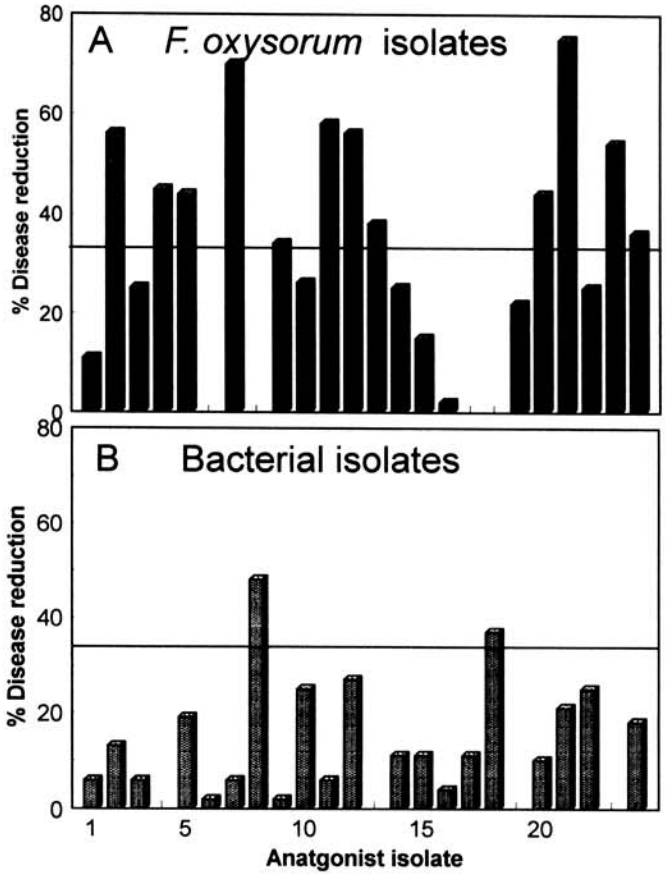

Fig. 3. Percent reduction of incidence of Fusarium wilt of watermelon in conducive field-soil screening tests by **A,** various nonpathogenic isolates of *Fusarium oxysporum* collected from suppressive soil and **B,** various bacterial isolates, including fluorescent pseudomonads, that were effective in reducing disease in microwave-treated soil. Horizontal line indicates the level of significant reduction of disease (LSD, $P = 0.05$). All bars extending above the line represent isolates that significantly reduced disease. Data for each isolate represent results from a single field-soil test. Average wilt in untreated control soil was 72.5%.

were more effective as a group than isolates from FGM soil, with average reductions of 21.9 and 11.6%, respectively. However, only specific isolates of *F. oxysporum* from suppressive soil were consistently effective in reducing Fusarium wilt in all field-soil tests (Table 2). Some isolates provided disease reduction comparable to suppressive soil (28% wilt and 62% reduction) over all tests. No isolates of fluorescent pseudomonads, actinomycetes, other bacteria, or other fungi resulted in significant disease reduction in multiple field-soil tests.

Several of these successful isolates also were tested in field soil with chlamydospore inoculum at rates lower than or comparable to the pathogen inoculum levels (50 to 200 CFU/g of soil). Significant reductions in disease were observed at antagonist inoculum levels as low as 50 chlamydospores per g of soil (Table 3).

Characteristics of successful antagonists. To evaluate the role of root colonization in suppression of disease, root colonization characteristics for isolates of each antagonist organism type that were successful in reducing disease were compared as a group with isolates that were not successful in reducing disease (Table 4). Isolates of *F. oxysporum*, whether effective or not effective in reducing disease in microwave-treated soil, in general, resulted in reduced root colonization by the OM pathogen compared to microwave-treated control soil and all bacterial isolates tested. Colonization by the pathogen in the presence of both groups of antagonist *F. oxysporum* also was comparable to the colonization observed in the suppressive soil control. There was no difference in colonization by the potential antagonist between effective and noneffective isolates of *F. oxysporum*. Colonization by *F. oxysporum* was higher in all antagonist treatments with *F. oxysporum* than in the suppressive soil controls. Effective bacterial isolates reduced colonization by the pathogen compared to noneffective isolates and the microwave-treated control soil groups. Wilt levels were comparable for effective antagonist groups and the suppressive soil controls, all of which were less than those for the noneffective isolates and microwave-treated control soil (Table 4).

Comparisons of internal root colonization for similar antagonist groupings in field-soil tests gave results similar to those in microwave-treated soils (Table 4). Colonization by the OM pathogen was lower in treatments with isolates of *F. oxysporum* than in those with noneffective bacteria and infested field-soil controls, but there was no difference between the effective and noneffective isolates of *F. oxysporum*. There was, however, a difference in colonization by other *F. oxysporum* between effective and noneffec-

TABLE 2. Isolates of *Fusarium oxysporum* most effective in reducing Fusarium wilt of watermelon in repeated antagonist screening tests with conidial inoculum

Isolate	MW-treated soil[x]		Field soil[y]	
	% Wilt	% Reduction	% Wilt	% Reduction
CS-2	25 a[z]	73 bc	23 a	65 d
CS-1	40 a	50 b	31 a	60 cd
CS-7	31 a	68 bc	32 a	51 b–d
CS-3	38 a	62 bc	35 a	52 b–d
CS-5	40 a	51 bc	33 a	48 bc
CS-11	25 a	62 bc	35 a	45 b
CS-6	31 a	69 bc	37 a	42 b
CS-31	10 a	89 c	38 a	40 b
CSMW soil control	81 b	0 a	...	...
CSS soil control	...	...	28 a	62 d
LFC soil control	...	...	73 b	0 a

[x] Microwave-treated (MW) suppressive Crimson Sweet (CSS) monoculture soil was infested with chlamydospores of the orange mutant (OM) pathogen strain of *F. oxysporum* f. sp. *niveum* at 200 CFU/g of soil, followed by incorporation of a conidial suspension of the antagonist at 10^4 CFU/g of soil. Percent reduction of disease was determined relative to the pathogen-infested control soil in each test.

[y] Field soil screening tests used a conducive field soil (LFC) infested with chlamydospores of the OM pathogen at 300 CFU/g of soil, followed by introduction of a conidial suspension of the antagonist at 10^4 CFU/g of soil. Values represent means of three similar tests combined for each isolate.

[z] Means within columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

TABLE 3. Percent reduction in incidence of Fusarium wilt of watermelon by selected isolates of *F. oxysporum*, using chlamydospore inoculum

Isolate	% Disease reduction			
	Experiment 1[x]			Experiment 2[y]
	50 CFU	100 CFU	200 CFU	50–100 CFU
CS-1	38*[z]	24*	24*	49*
CS-6	43*	16*	8	68*
CS-24	5	15*	24*	72*
CS-40	28*	8	15*	...
CS-5	...	...	...	75*

[x] Chlamydospore inoculum added to conducive field soil at 50, 100, and 200 CFU/g of soil prior to planting. Pathogen inoculum added at 100 CFU/g of soil. Watermelon cultivar Florida Giant (susceptible) was planted. Isolate × inoculum-dose interaction was significant ($P < 0.05$).

[y] Chlamydospore inoculum added to conducive field soil at 50 CFU/g of soil prior to planting for isolates CS-1 and CS-5 and at 100 CFU/g of soil for isolates CS-6 and CS-24. Inoculum levels were selected based on preliminary tests with the isolates. Pathogen inoculum was added at 100 CFU/g of soil. Watermelon cultivar Crimson Sweet (moderate resistance) was planted.

[z] Means followed by asterisks represent significant ($P < 0.05$) reduction of disease according to Fisher's LSD test.

TABLE 4. Effect of antagonists on internal colonization of watermelon roots by *Fusarium oxysporum* f. sp. *niveum* and other *F. oxysporum* isolates in microwave-treated suppressive soil and field soil as classified by antagonist groupings

Treatment group[w]	No. of isolates[x]	No. of colonies/100 cm of root[y]		% Wilt
		OM pathogen	*F. oxysporum*	
Microwave-treated soil				
Effective *F. oxysporum*	25	4.33 a[z]	10.38 c	30 a
Noneffective *F. oxysporum*	8	5.28 a	10.80 c	61 b
Effective bacteria	10	10.28 b	1.92 a	34 a
Noneffective bacteria	11	12.97 c	0.80 a	61 b
MW-treated soil control	...	14.38 c	0.37 a	73 c
Suppressive soil control	...	4.73 a	6.86 b	35 a
Field soil				
Effective *F. oxysporum*	14	9.15 b[z]	5.39 a	39 b
Noneffective *F. oxysporum*	12	8.41 b	8.33 b	68 c
Effective bacteria	2	8.91 bc	3.28 a	47 b
Noneffective bacteria	6	12.79 c	4.95 a	75 c
Field soil with pathogen	...	14.09 c	4.94 a	73 c
Field soil with no pathogen	...	0.00 a	4.41 a	0 a

[w] Data from a number of individual isolates were grouped together according to organism type and effectiveness in reducing disease ($P < 0.05$) in microwave-treated soil.

[x] Values represent the number of isolates within each treatment group from which individual colonization data were compiled.

[y] Roots of 4-week-old plants were washed, surface-disinfested in 0.5% sodium hypochlorite for 1 min, rinsed, and embedded intact in Komada's medium. Colonization of roots by the orange mutant (OM) pathogen strain of *F. oxysporum* f. sp. *niveum* and all other *F. oxysporum* strains was determined. Values represent average colonization for all isolates in each group. Pathogen inoculum was added as chlamydospores at 200 CFU/g of soil.

[z] Means within columns followed by the same letter for each soil type are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

tive isolates of *F. oxysporum*, with higher colonization for the noneffective group than for any other group. Colonization data for individual isolates followed the same trends as for the overall groups in both soils.

There was no effect of isolation method on the recovery of effective antagonist isolates. Isolates collected from the rhizosphere, rhizoplane, or surface-disinfested roots all demonstrated comparable levels of effectiveness for all organism groups. Likewise, isolating from roots grown in soil microwave-treated for 0, 30, or 60 s/kg had no effect on the recovery of effective isolates.

Vegetative compatibility analyses were conducted on 42 isolates of *F. oxysporum*, representing 24 effective and 18 noneffective antagonists, with 30 isolates from CSS soil and 12 from FGM soil. No isolates were compatible with pathogenic VCGs nor were they compatible with each other. There were no common VCGs identified among any of the isolates tested, whether effective or not effective as antagonists.

ISR. The split-root technique employed in this study was successful in separating the pathogen from potential antagonists in suppressive soil. Individual control plants as well as split-root plants showed no cross-contamination on roots between the two pots. Treatment with an antagonistic isolate of *F. oxysporum*, which was highly effective in reducing disease in field-soil tests, resulted in significantly lower disease incidence, based on the percentage of plants visually showing wilt symptoms, than conducive soil treatments (Table 5). The suppressive soil treatment resulted in somewhat lower ($P < 0.1$) disease incidence than the conducive field-soil treatment but was not statistically significant at $P < 0.05$. However, when disease was assessed by the percentage of plants with the OM pathogen present in the vascular tissue of the above-ground stem sections, there were no differences among any of the treatments; nearly all plants had systemic infection regardless of treatment. In a repetition of this experiment, treatment with a different antagonist isolate also resulted in lower disease levels than the microwave-treated soil, but the suppressive-soil treatment did not significantly reduce disease levels.

DISCUSSION

The wide discrepancy in effectiveness among individual nonpathogenic isolates of *F. oxysporum* indicates that specific antagonistic isolates are responsible for disease suppression in these soils rather then a general population response attributable to all nonpathogenic strains of *F. oxysporum*. A larger proportion of effective antagonistic isolates of *F. oxysporum* was found in CSS than in FGM soil, although even in suppressive soil effective antagonists accounted for a relatively small proportion (estimated at from 16 to 39% in CSS soil versus 3 to 12% in FGM soil) of the total population of *F. oxysporum*. Effective antagonist isolates evidently possess specific traits, absent from the majority of isolates, that make them successful in reducing disease. The effectiveness of isolates of *F. oxysporum*, as well as the failure of any other fungal isolate or individual bacteria to effectively reduce disease in these tests, is similar to the findings of Alabouvette and coworkers (1,2, 27,42) using similar screening tests with the suppressive soils of the Chateaurenard region of France.

Testing of the isolates in microwave-treated suppressive soil was effective as a first step in the antagonist screening process. Using this biological assay, the organisms that have some antagonistic activity toward the pathogen or disease development were identified (1,2,27). This provided a substantial pool of potential antagonists, yet it succeeded in eliminating 40 to 90% of the original isolates from each organism category that would not be active in suppression. However, even with this preliminary screen, only 35% of the *F. oxysporum* isolates and ~7% of all other organisms that were effective at reducing disease in microwave-treated soil also were effective in field soil. The restoration of suppression to this microbiologically disrupted system did not necessarily translate into a corresponding role in disease suppression in field soil.

Other organisms that demonstrated some antagonistic activity in microwave-treated soil may play some role in disease suppression, however. Although these organisms may not be effective as antagonists individually, they may enhance the overall suppressive effect in combination with other groups of antagonistic organisms or by providing a background of general suppression in which the specific suppression by *F. oxysporum* operates (2,6,27). Fluorescent pseudomonads enhance the suppression provided by antagonistic *F. oxysporum* in some soil systems (25,26,33). Although some individual isolates of *F. oxysporum* appeared to be as effective as the untreated suppressive soil in reducing disease, most isolates were not, indicating that other organisms may contribute to suppression. Differences were observed between populations of various microorganisms in CSS and FGM soils in previous studies (21,22), and some of these differences may be involved in the overall suppressive response of CSS soil.

Selected isolates of *F. oxysporum* were effective both as conidial and chlamydospore inocula. Chlamydospore inoculum was effective at levels as low as 50 to 100 CFU/g of soil, which was comparable to or below the inoculum levels of the pathogen. This is in contrast to other reports of antagonism by isolates of *Fusarium* spp. obtained from naturally occurring suppressive soils, which typically require antagonist population levels 10 to 100 times greater than those of the pathogen to be effective (1,3,4,13,34). The mechanism of action of most of these other isolates is competition, and thus, they require inoculum levels substantially greater than the pathogen to successfully prevent pathogen infection. Biocontrol agents that are effective at lower inoculum levels require less inoculum, may be easier to implement and maintain, and may provide more consistent control under a variety of environmental conditions, because control is not as dependent on fluctuations in population size. Antagonist effectiveness did not appear to be closely related to population levels in the current study.

Vegetative compatibility has been used as a means of subdividing natural populations of *F. oxysporum*, and isolates within a VCG often share specific traits, such as virulence, colony size, and isozyme patterns (7,8,20,35). Extreme VCG diversity has been observed among nonpathogenic isolates of *F. oxysporum* by others (8,12). Similarly, in the preliminary screening done in this study, a large

TABLE 5. Development of Fusarium wilt in split-root watermelon plants with one-half of each root system exposed to the pathogen and the other half exposed to conducive- and suppressive-soil treatments

Treatment soil[x]	Experiment 1[y]		Experiment 2
	% Wilt	% Systemic infection	% Wilt
Microwave-treated suppressive soil	81 a[z]	92 a	65.3 a
Conducive field soil	87 a	100 a	57.0 ab
Suppressive soil	65 ab	100 a	40.8 a–c
F. oxysporum antagonist (CS-6)	...	...	34.7 bc
F. oxysporum antagonist (CS-7)	43 b	87 a	20.8 c

[x] The orange mutant (OM) pathogen strain of *F. oxysporum* f. sp. *niveum* was added to one side of a dual-pot setup at 400 CFU/g of soil, and the treatment soil filled the other pot. Antagonist treatment (CS-7 or CS-6) consisted of a root-dip of one-half of the root system in a conidial suspension of 10^6 CFU/ml prior to transplanting. Plants were grown for 5 weeks.

[y] Visual symptoms were assayed as the percentage of plants showing severe wilt symptoms. Systemic infection was represented by the percentage of plants in which the OM pathogen was present within the vascular tissue. Determinations of systemic infection were made by plating surface-disinfested stem sections on Komada's medium.

[z] Means within columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

number of VCGs were present among the isolates tested, with no two isolates belonging to the same VCG. Thus, the traits related to the ability to reduce disease apparently are not associated with specific VCGs and are common to many VCGs. Due to this diversity, vegetative compatibility was not a useful tool for identifying or differentiating effective antagonists from other isolates of *F. oxysporum*.

The most prevalent theories explaining antagonism by nonpathogenic *F. oxysporum* are saprophytic competition at or near the root surface (1,4,9,24,27), parasitic competition for infection sites on the root (39), and induced resistance to pathogen infection by prior colonization (19,31,32,40). Mandeel and Baker (28) demonstrated that these mechanisms are not necessarily exclusive of one another. Indeed, they observed that all three mechanisms were active in a single isolate of *F. oxysporum*. Saprophytic competition for carbon and iron is known to be important in the suppression of Fusarium wilt in Chateaurenard soils (1,4,9,24,27) and soils of the Salinas Valley of California (11,37,38,41). Induced resistance, although demonstrated with individual *F. oxysporum* isolates, has not been shown previously to be a mechanism of suppressive soils (31).

In our tests, colonization data indicated that reduction of disease was not related to the ability of an antagonist to extensively colonize roots or to reduce colonization by the pathogen. Although colonization of the root by the antagonist may be necessary for antagonism to occur, colonization alone did not necessarily result in lower disease. Schneider (39) also observed that many isolates of *F. oxysporum* were capable of colonizing roots, but only some isolates were effective antagonists. In Schneider's (39) tests, reduction in root colonization by the pathogen was directly related to antagonist effectiveness. In our study, reduction in root colonization by the pathogen caused by the antagonist was not sufficient to reduce wilt in either microwave-treated or field soil. The relative ability of an antagonist to compete with the pathogen for colonization of the roots (infection sites) was not related to its effectiveness as an antagonist. This is direct evidence against parasitic competition for infection sites on the root as the primary mechanism of suppression in this soil. These results, in addition to colonization data from previous studies (21,22), also suggest that saprophytic competition is not the primary mechanism of disease suppression in these soils.

Several studies have shown that nonpathogenic or avirulent strains of *F. oxysporum* applied to roots can protect various hosts from Fusarium wilt when challenged by a virulent strain (10,14,29,31, 32). This response also has been observed for Fusarium wilt of watermelon (5,30,40). Several researchers (19,28,32) have demonstrated that protection was a result of ISR caused by previous infection by nonpathogenic *F. oxysporum*. However, many other studies suggesting induced resistance as the mechanism of control do not adequately distinguish this response from competition at the roots, because both antagonist and pathogen treatments are normally applied to the same roots (31). For this reason, a split-root technique was used in this study to assay for ISR by *F. oxysporum*.

Split-root experiments demonstrated that selected isolates of *F. oxysporum* from suppressive soil induced systemic resistance to Fusarium wilt. There was evidence of a significant reduction or delay in symptom development even though the antagonist did not prevent systemic infection by the pathogen. Plants treated with suppressive soil or individual antagonists often showed partial wilt, with the pathogen present only in the vascular bundles on one side of the plant. This may indicate limited movement of the pathogen in these plants. Maraite (29), working with Fusarium wilt of muskmelons in similar split-root experiments, as well as other researchers (5,10) observed that induced resistance by nonpathogenic *F. oxysporum* resulted primarily in a reduction or delay in disease development and that this effect could be overcome by increases in pathogen inoculum density (30). Hillocks (15), working with induced resistance to Fusarium wilt of cotton, concluded the mechanism was vascular occlusion induced by a nonpathogenic strain of *F. oxysporum* that limited the movement and reduced populations of the pathogen within vascular tissue. Thus, the mechanism of induced resistance probably involves reducing or restricting the extent of pathogen colonization or movement within vascular tissue rather than prevention of infection or initial colonization of roots.

The slight reduction in disease observed in both ISR tests for the suppressive-soil treatment indicates that induced resistance may play a role in the activity of the suppressive soil but that it does not totally explain the suppressiveness. Thus, other mechanisms also must be responsible for the effectiveness of the suppressive soil. This additional suppression may be due to competition provided by antagonistic *F. oxysporum* or other organisms, such as fluorescent pseudomonads, or to a combination of several mechanisms.

The results of this study, along with our previous work (21,22), indicate that indigenous isolates of *F. oxysporum* not pathogenic on watermelon are the dominant antagonists and primary organisms responsible for suppression of Fusarium wilt of watermelon in Crimson Sweet suppressive (CSS) monoculture soil, although other organisms may contribute to suppression. Currently, research is continuing to develop these antagonists as biocontrol agents, with further investigations into their mechanisms of action, the conditions under which they are effective, and ways to improve their consistency and level of effectiveness. Preliminary field tests with selected isolates have shown promising results (23). Several of these isolates have shown comparable effectiveness in reducing Fusarium wilt of other vegetable crops, including tomato and muskmelon, in preliminary tests (R. P. Larkin and D. R. Fravel, *unpublished data*).

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