Ecology of *Fusarium oxysporum* f. sp. *niveum* in Soils Suppressive and Conducive to Fusarium Wilt of Watermelon

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ABSTRACT

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Population dynamics and chlamydospore germination of *Fusarium oxysporum* f. sp. *niveum*, as well as colonization of watermelon roots by *F. oxysporum*, were monitored in relation to other microorganism populations and the incidence of Fusarium wilt in four soils representing different suppressive and conducive conditions. The soils consisted of an induced suppressive soil developed through monoculture to watermelon cultivar Crimson Sweet, a nonsuppressive monoculture soil, a conducive fallow soil, and the suppressive soil rendered conducive by microwave treatment. An orange-colored mutant isolate of the pathogen, comparable to the wild-type in growth, pathogenicity, and root colonization, was used to differentiate the pathogen from indigenous populations of *F. oxysporum* in the field soils. Pathogen populations remained stable in the monoculture soils over a 6-mo period, but increased somewhat initially and remained at higher levels when added to conducive soils. Suppressiveness was not associated with inhibition of pathogen chlamydospore germination. There were no differences among the field soils in pathogen chlamydospore germination with glucose amendments of 0–1.0 mg/g of soil. Populations of general bacteria, actinomycetes, and fluorescent pseudomonads, both in soil and on watermelon roots, tended to be greater in suppressive than in conducive field soils, whereas fungal populations were greatest in conducive field soil. Root colonization by introduced *F. o. niveum*, indigenous *F. oxysporum*, or other microorganism groups was not consistently related to suppressiveness, suggesting that specific antagonistic strains rather than general populations of microorganisms may be involved in suppression.

Additional keywords: biological control, *Citrullus lanatus*, soil microbiology.

Fusarium wilt of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), caused by *Fusarium oxysporum* f. sp. *niveum* (E. F. Sm.) Snyd. & Hans., occurs throughout the world and is often a limiting factor in watermelon production. Soils that are naturally suppressive to Fusarium wilt of numerous crops are known to occur in many regions of the world (2,6,40,52). In these soils, disease does not readily develop, even though the pathogen and susceptible hosts are present (6,40). Among the most well-known and studied examples of Fusarium wilt-suppressive soils are those of the Chateaurenard region of France (2,4,30) and in the Salinas Valley in California (38,39,45,46,49,53). The cause of disease suppression in these and many other such soils has been determined to be biological in origin and has been associated with inhibition of chlamydospore germination and reduced saprophytic growth of the pathogen (2,4,21,22,30,38,39). These soils generally have many characteristics in common, including their physical-chemical makeup (high pH, organic matter, and montmorillonite clay content), transmissibility of suppressiveness to certain other soils, and effectiveness against a number of *F. oxysporum* f. sp. (2,4,6,30,40).

Numerous organisms have been reported as causing or contributing to this suppressiveness in various soil systems, most notably nonpathogenic *Fusarium* spp. (2,4,30,41,50) and fluorescent *Pseudomonas* spp. (7,8,38,39,49), but also species of *Arthrobacter* (45,47), *Alcaligenes* (54), *Trichoderma* (28,29,32), *Penicillium* (28,32), *Bacillus* (54), *Serratia* (47,48), *Hafnia* (48), and others have been implicated in various studies. In addition, a general suppression provided by a large, diverse population of antagonistic bacteria and actinomycetes supported by the physical characteristics of many of these soils also appears to be important (2,4,6,30,38,39).

In recent years, Hopkins and co-workers (18) have reported the development of a soil suppressive to Fusarium wilt of watermelon in the field that was induced by monoculture to a particular watermelon cultivar (Crimson Sweet). This soil has maintained extremely low levels of wilt in the field for over 10 seasons and demonstrates suppressiveness in greenhouse tests in which additional pathogen inoculum is added and susceptible cultivars are planted (18). This suppression differs from most other Fusarium wilt-suppressive soils, not only in its apparent cultivar-specific induction, but also in that it occurs in a soil normally conducive to disease (sandy, low pH, low organic matter content) and is not readily transmissible to other soils (18). The suppression has

been determined to be of biological origin, but other characteristics, as well as the organisms and mechanisms responsible for the suppression, have not yet been identified.

Because of the complex nature of soils suppressive to plant disease, it is important to study the overall conditions, processes, and mechanisms which make them suppressive (6,40,52). A thorough understanding of the ecological interactions of the pathogen and other microorganisms may be vital for the effective utilization of specific antagonists from these soils as biological control agents. Through the analysis of environmental differences in suppressive and conducive soils, insight may be gained into the effect of the suppressive soil on the pathogen and how suppression occurs.

The unique development of suppressiveness in the Crimson Sweet monoculture soil made it possible to make comparisons with similar conducive soils and evaluate some ecological characteristics that may be related to suppressiveness. Using four soils to represent different suppressive and conducive conditions, the objectives of this study were to monitor the population dynamics and chlamydospore germination of *F. o. niveum* and to evaluate the colonization of watermelon roots by this pathogen and native *F. oxysporum* isolates in relation to other microorganism populations and the incidence of Fusarium wilt. A preliminary report of portions of this work has been published (25).

MATERIALS AND METHODS

Soils. Four soils were used throughout this research to represent different conditions of suppressiveness and conduciveness to Fusarium wilt. All of the soils were collected from field plots and adjacent areas of the experimental farm at the Central Florida Research and Education Center, Leesburg, FL. All are of the Apopka Fine Sand soil series, which are loamy, siliceous, hyperthermic Grossarenic Paleudults. They have a pH of 6.0–6.5, are low in organic matter (<1%), clay content (<3%), cation exchange capacity (4–6 meq/100 g of soil), and available water capacity, and have similar physical and chemical characteristics (17). They differ only in their cropping history and the resulting biology. Soils were collected in large buckets, sieved through a 0.2-cm screen, and stored in plastic bags for up to 4 mo before use. The soils are designated as follows.

1) Crimson Sweet suppressive (CSS) monoculture soil is the Fusarium wilt-suppressive soil developed through monoculture to moderately resistant watermelon cultivar Crimson Sweet. Total populations of *F. oxysporum* in the soil average $1–2 \times 10^3$ colony-forming units (cfu)/g of soil, but the watermelon wilt pathogen apparently accounts for a relatively small proportion of this total (10–30%) (19, D. L. Hopkins and R. P. Larkin, *unpublished data*). Addition of substantial amounts of the pathogen to this soil did not significantly increase disease incidence (18).

2) Florida Giant monoculture (FGM) soil is a nonsuppressive soil from the same field as CSS, but it has been monocultured to the susceptible cultivar Florida Giant. High levels of wilt occur in the field, but the soil is similar to CSS in its biology and overall effects due to prolonged monoculture. Total populations of $1–2 \times 10^3$ cfu/g of soil of *F. oxysporum* are similar to those in CSS soil, with pathogen populations comprising a proportion (10–40%) of the total population of *F. oxysporum* similar to those in CSS soil (19, D. L. Hopkins and R. P. Larkin, *unpublished data*). This soil has been called nonsuppressive rather than conducive because the addition of moderate amounts of the pathogen causes little change in disease, suggesting that this soil does have some biological buffering capacity against the pathogen and is not as conducive as fallow and other soils (18).

3) Leesburg fallow conducive (LFC) soil is from the same vicinity as the monoculture soils, but from an area that had not been planted to watermelon or any other crop. This represents the natural soil of the area before cultivation of watermelon. Total populations of *F. oxysporum* are lower ($0.2–1.0 \times 10^3$ cfu/g of soil) than those in the other soils, and the pathogen is generally not detectable; the addition of small quantities of the pathogen causes substantial disease.

4) Microwave-treated Crimson Sweet suppressive (CSMW) soil is the CSS soil rendered conducive by microwave-irradiation (2,450 MHz, 700 W) for 2 min/kg of soil at a matric potential of −10 J/kg. This treatment was sufficient to remove all suppressive characteristics and eliminate all *F. oxysporum* and most other fungi, yet leave a large bacterial biomass (11,26).

Infestation of soils with *F. o. niveum* and assay of Fusarium wilt. Isolates of *F. o. niveum* were obtained from naturally infected watermelon plants in a previous study (18). For most tests, race 1 isolate FG85-1 was used (26). Chlamydospores of the pathogen were used as inoculum to simulate natural conditions in the soil. Initial cultures were grown for 7 days in a mineral salts solution (33) and homogenized in a blender. This liquid culture, consisting mainly of chopped mycelia and microconidia, was then added to autoclaved (60 min/kg) soil. The soil was moistened, mixed, and allowed to dry. After 4–8 wk, primarily chlamydospores remained, and dilution plate counts were made using Komada's (23) selective medium for *F. oxysporum* to determine their density in soil as previously described (26). This stock inoculum soil was then mixed with the soil to be infested to produce the desired inoculum level (~200 chlamydospores per g of soil unless otherwise stated).

Watermelon seeds of the susceptible cultivar Florida Giant and the moderately resistant cultivar Crimson Sweet were planted in pots of the infested soil (four to six plants in each of four to six replicate pots) in the greenhouse. Plants were maintained at 20–30 C and grown for 4 wk on a greenhouse bench. 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 (23) medium. Wilt was expressed as the percentage of diseased plants over the 4-wk period.

Production and characterization of orange mutant pathogen isolates. To distinguish *F. o. niveum* added to field soils from indigenous isolates of *F. oxysporum*, an ultraviolet light-induced, orange-colored, mutant isolate of the pathogen was produced and characterized for use as a marker organism (9,36,41). Microconidia from 5- to 7-day-old potato-dextrose agar (PDA) cultures of a race 1 isolate (FG85-1) of *F. o. niveum* were suspended in water and adjusted to 10^6 spores per milliliter. A petri dish containing 10 ml of this suspension was exposed to ultraviolet light (General Electric G8T5 germicidal lamp, General Electric Co. Fairfield, CT) at a distance of 10 cm for 20 s in a dark chamber; approximately 95% of the spores were killed. Survivors were plated (200–500 per plate) on a sorbose-based medium, which consisted of the following ingredients per liter of water: 20 g of agar; 20 g of sorbose; 2 g of asparagine; 1 g of K_2HPO_4; 0.5 g of KCl; 0.5 g of $MgSO_47H_2O$; 0.5 ml of tergitol NP-10; and 0.2 ml of a trace element solution composed of 5 g of citric acid; 5 g of $ZnSO_47H_2O$; 4.75 g of $FeSO_47H_2O$; 1 g of $Fe(NH_4)_2(SO_4)_26H_2O$; 0.25 g of $CuSO_45H_2O$; 0.05 g of $MnSO_4$; 0.05 g of H_3BO_4; and 0.05 g of Na_2MoO_4 in 95 ml of water. Orange mutants were readily distinguished from the wild type by their distinct orange pigment after 6 days.

Growth of the orange mutant (OM) isolates was compared with that of the wild-type parent isolate by measuring radial colony growth and microconidium production on PDA as well as weight increase of mycelia in a liquid medium. Radial growth measurements were made after 3, 5, and 7 days at 26 C. After 7 days, 10 ml of sterile water was added to the plates, and the surface of the agar was gently scraped to loosen the mycelia and conidia. Conidial counts of the resulting suspension were made using a hemacytometer. Mycelial PDA blocks also were transferred to flasks containing 30 ml of liquid culture medium (33) and incubated at 26 C for 5 days. Mycelial mats were harvested on filter paper, washed twice with deionized water, and dried at 80 C for 24 h before weighing. Each test was conducted at least twice with four replications per isolate.

Pathogenicity of the OM isolates was tested and compared with that of the wild-type parent isolate in microwave-treated soil and a conducive field soil using both conidia and chlamydospores as inoculum. Conidia were collected from 5- to 7-day-old PDA cultures and added to the soil at the rate of 5×10^3

cfu/g soil. Chlamydospores were added to soil at 200 cfu/g soil. Watermelon seeds were planted in four to six replicate pots (five seeds per 7.5-cm pot), and wilt was assayed as described above.

Root colonization by the OM isolates was compared with that of the wild-type parent isolate in microwave-treated soil infested with the pathogen. Watermelon seeds were planted (four per pot, four replicate pots per isolate) in soil infested with 5×10^3 conidia per gram of soil or 200 chlamydospores per gram of soil and grown for 3 wk. Roots were gently removed from the soil and washed under running water to remove adhering soil particles. Roots were blotted dry, weighed, put in sterile water, and shaken at 150 rpm for 20 min. The resulting suspension and a 1:10 dilution were plated on Komada's (23) medium, and colonies were counted after 5–7 days. Counts were converted to colony-forming units per gram of root fresh weight. In another technique, roots were washed, separated, cut into sections, and plated directly by laying the pieces lengthwise on Komada's (23) medium and then observed for the number of orange mutant colonies per 10 cm of length of root.

Survival and population dynamics of *F. oxysporum* in field soils. Chlamydospore inoculum of an OM isolate of the pathogen (FG-OR3) was added to each of the four soils at rates ranging from $2–10 \times 10^2$ cfu/g soil. Infested soil was maintained either with the matric component of soil water potential (matric potential) held constant at −100 or −10 J/kg or under a fluctuating moisture regime of alternating wetting and drying cycles. For maintaining a constant matric potential, infested, air-dried soil was moistened with deionized water to attain the desired matric potential as determined by soil moisture release curves calculated for each soil. Subsamples of 50 g of each infested soil were kept in small, weighed, plastic screwtop containers. The containers were placed, with tops loosened, in a moist chamber and incubated at 28 C. Water was added to the containers weekly to replenish the moisture content and maintain their original weights. Weekly variation of less than 1% moisture content was observed. Infested soil samples for the fluctuating moisture regime tests were put in plastic pots in the greenhouse, saturated with water, and allowed to slowly dry. After about 2 wk, the soil was resaturated, and this wetting and drying process was repeated throughout 6 mo. Periodically, the soils were stirred with a spatula, and 5-g soil subsamples were taken from these (during the dry phase of the cycle) and used to determine the soil populations of the OM pathogen as well as indigenous *F. oxysporum* by soil dilution plating on Komada's (23) medium as previously described. Populations were monitored over a 6-mo period and determined as colony-forming units per gram of air-dried soil. Tests were made using various initial inoculum densities and consisted of three replications of each soil, with two samples taken per replication. The tests with fluctuating moisture regime and −100 J/kg constant moisture were repeated, whereas the −10 J/kg test was only conducted once.

Chlamydospore germination. Germination of chlamydospores of the pathogen in the four soils was assessed by using the buried membrane filter technique of Adams (1), with modifications by Alabouvette et al (3,5). Chlamydospores were produced by growing the fungus on carnation leaf agar and suspending the macroconidia in sterile water. The suspension was incubated in the dark at 26 C for 2 wk, at which time most macroconidia had converted to chlamydospores. Chlamydospore suspensions were adjusted to $2–3 \times 10^4$ cfu/ml, and 1 ml of this suspension was deposited on a 25-mm millipore membrane filter (type HA 0.45 μm) with a grid on one side, by vacuum filtration.

A glucose solution was added to 100-g subsamples of the test soils at the rates of 0, 0.1, 0.2, 0.4, or 1.0 mg/g soil to give a final matric potential of −10 J/kg. The treated soils were placed in 5.5-cm-diameter plastic pots, and the chlamydospore-containing membrane filter was buried in the soil by making a slit with a spatula, gently inserting the filter, and covering the filter with soil. The pots were incubated in a moist chamber for 24 h at 25 C. Filters were then gently removed, rinsed in deionized water, stained with trypan blue lactophenol (1), steamed at 70 C for 20 min, washed with clear lactophenol by vacuum filtration, and mounted in glycerin on microscope slides. Filters were subdivided into six sectors, and at least 100 chlamydospores were counted per sector at a magnification of 200×. Chlamydospores were observed for germination and germ tube length. Germination tests were conducted on representative isolates of races 1 and 2, and tests were performed at least three times for both isolates.

Root colonization by *F. oxysporum*. Watermelon cultivars Florida Giant and Crimson Sweet were planted in the four soils infested with the OM pathogen at a rate of about 200 cfu/g of soil and grown 2–3 wk in the greenhouse. Whole root samples were gently removed from the pots, and adhering soil was removed under running tap water. In initial experiments regarding surface colonization, roots were not treated further. In later experiments regarding internal colonization, roots were surface-disinfested in 0.5% sodium hypochlorite for 1 min and rinsed in deionized water. All roots were then placed in empty, sterile petri plates and covered with sterile water. The roots were separated and teased apart with a dissecting needle. The water was then poured off, and the intact root system was embedded in agar by pouring molten Komada's (23) medium cooled to 45 C into the plate covering the entire root. Plates were incubated at 28 C, and the colonies emerging from the root were counted 3–7 days later. Colonies of the OM pathogen, indigenous *F. oxysporum*, and other fungi easily could be differentiated and quantified. In this way, not only numbers, but the spatial arrangement of the pathogen in relation to other fungi, could be observed.

Lengths of the plated root systems were estimated by a modified line intersect method (51). The plates containing the roots were placed on a 0.5-cm grid, and the number of intersections of a root with a grid line was counted in both the vertical and horizontal directions over the entire plate. Root lengths in centimeters were determined by multiplication of the total intersections by a conversion factor of 0.393. Root systems ranged from 50 to 200 cm per plate. Colonization was expressed as number of colonies per 100 cm of root. Each experiment was repeated and consisted of four replications of four to six root systems each.

Soil and root microorganism populations. Estimates of populations of soil and root microorganisms were made using standard dilution-plating procedures on various general and selective culture media. Bacterial populations (rapid-growing, aerobic organisms capable of growing readily in culture) were estimated using nutrient agar and 1:10 strength tryptic soy agar. Actinomycetes were selected for using alkaline water agar, pH 10.5 (15). Populations of fluorescent pseudomonads as well as certain other pseudomonads were determined on selective King's medium B containing penicillin, cyclohexamide, and novobiocin (37). Bacterial plates were incubated at 26 C for 3–4 days. Plates of King's medium B were examined under ultraviolet light for colonies producing diffusible fluorescent pigments. Colonies with a similar pseudomonadlike morphology not producing fluorescent pigment also were counted. Actinomycete plates were incubated for 7–10 days, and total colonies were counted. Fungal populations (primarily rapid-growing, spore-forming organisms) were determined on PDA containing 1 ml of tergitol NP-10 and 50 mg of chlortetracycline per liter of medium. Plates were incubated at 26 C for 5–6 days, and total colonies were counted.

A series of 1:10 dilutions of the initial soil suspensions were made and 0.1-ml aliquots of the appropriate dilutions were spread-plated on the various agar media. Population densities of rhizosphere and rhizoplane microorganisms were estimated from roots of 3-wk-old watermelon plants. Roots were gently removed from soil, and loosely adhering soil was shaken free. Roots were weighed, placed in sterile water in a 1:50, w/v, dilution, and either shaken on a rotary shaker at 200 rpm for 20 min or put in a sonicator (Branson ultra-sonic cleaner model B-22-4, Branson Ultrasonics Corp., Danbury, CT) for 5 min, depending on the experiment. Appropriate 10-fold dilutions of the resulting suspensions were plated on the various agar media. In some tests, rhizoplane organisms remaining on the root after sonication also were estimated. Roots were removed from the suspension after the sonication procedure, rinsed in sterile water, and triturated in a mortar and pestle. Final dilutions were plated as with the

others. Populations for all organisms were expressed as log colony-forming units per gram of soil. For most experiments, four replications of four plates each were used for each soil or root treatment. All experiments were conducted twice.

Selective elimination of microorganisms by microwave irradiation. Effects of various exposure times of field soils to microwave irradiation on the level of disease suppression (wilt), microorganism populations, and root colonization by *F. oxysporum* were assessed. Soils were adjusted to a matric potential of −10 J/kg and microwave-irradiated (2,450 MHz, 700 W) for 0, 30, 60, or 90 s/kg soil. These levels were chosen after preliminary tests (levels ranging from 0–150 s/kg at 15-s intervals) showed disease suppression was eliminated in CSS soil after a microwave exposure of 90 s/kg or longer. Chlamydospore inoculum of the OM pathogen was added to the treated soils at the rate of 200 cfu/g soil. Watermelon cultivars Crimson Sweet and Florida Giant were planted in the soils (four replicate pots of 10 plants each for each treatment) and grown for 4 wk. Wilt was assessed, and root microorganism populations and colonization were determined as described above.

Statistical analyses. Statistical analyses were conducted using the general linear models procedures of the Statistical Analysis Systems version 6.04 (SAS Institute, Inc., Cary, NC). Experimental design for most tests were variations on a randomized complete block, generally with four to six replications and a factorial treatment structure. Experiments were analyzed using standard 1- to 3-factor analysis of variance (ANOVA) with interactions. Significance was evaluated at $P < 0.05$ for all tests. Factor and interaction sums of squares were partitioned into single degree of freedom planned orthogonal contrasts, as class comparisons

TABLE 1. Comparison of selected orange mutant isolates with the wild-type parent isolate (FG85-1) of *Fusarium oxysporum* f. sp. *niveum* for growth characteristics, root colonization, and pathogenicity

Isolate	Radial growth[u] (mm)	Conidial production[v] (cfu × 10^6/ml)	Mycelial mass[w] (mg)	Root colonization[x] Rootwash (cfu × 10^3/g)	Root colonization[x] Direct (cfu/10 cm)	Pathogenicity[y] (% wilt) MW-treated soil	Pathogenicity[y] (% wilt) Field soil
FG85-1	38.9[z]	1.50 a	96	3.7	5.9	92	69
FG-OR3	38.0	3.52 c	95	4.6	9.7	91	69
FG-OR6	36.9	1.75 ab	95	4.3	...	81	62
FG-OR8	37.9	2.13 b	84	8.2	9.9	86	78

[u] Radial growth was measured on potato-dextrose agar (PDA) plates after 7 days at 26 C (average of three experiments, each with four replicate plates per isolate).

[v] Conidial production was estimated from 10-ml conidial suspensions made from 7-day-old PDA cultures (average of two experiments with four replications per isolate).

[w] Isolates were grown in liquid medium (33) for 5 days at 26 C. Means represent the dry weight of mycelial mats harvested on filter paper (average of two experiments with four replications per isolate).

[x] Weighed root samples (four per isolate) of cultivar Florida Giant were shaken in sterile water for 20 min, and the rootwash suspension was dilution-plated on Komada's (23) medium. For direct-plating, root samples were washed, separated, cut into sections, and plated lengthwise. Chlamydospore inoculum of 200 colony-forming units (cfu)/g soil was used in all tests.

[y] Pathogenicity was measured as the incidence of Fusarium wilt on cultivar Florida Giant in a microwave (MW)-treated soil (2 min/kg of soil at −10 J/kg) and a conductive field soil. Chlamydospore inoculum of 200 cfu/g of soil was used in all tests (average of three tests in microwave-treated soil and two tests in field soil using four replicate pots of five plants per test).

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

TABLE 2. Results of repeated measures analysis of variance for the population dynamics of *Fusarium oxysporum* f. sp. *niveum* over time in soil in which the matric component of soil water potential (matric potential) was held constant at −100 or −10 J/kg or was under a fluctuating moisture regime of wetting and drying cycles (data shown in Fig. 1)

Source of variation[x]	Soil moisture conditions[y] −100 J/kg df	−100 J/kg Sum of squares	−10 J/kg df	−10 J/kg Sum of squares	Wet/Dry cycles df	Wet/Dry cycles Sum of squares
Soil	3	1,352.9**[z]	3	1,420.8**	2	2,010.2**
CSS vs. FGM	1	6.0*	1	1.7	1	183.7
LFC vs. CSMW	1	536.0**	1	451.7**		...
Monoculture vs. conducive	1	810.8**	1	967.4**	1	1,826.5**
Error (soil)	12	10.3	20	281.2	9	372.6
Time (days)	5	576.5**	3	665.9**	5	2,136.8**
Linear	1	35.2**	1	85.4**	1	1,030.4**
Quadratic	1	205.8**	1	293.5**	1	0.1
Cubic	1	182.0**	1	287.5**	1	235.2**
Quartic	1	153.3**		...	1	437.1**
Residual	1	0.1		...	1	434.0**
Soil × time	15	928.6**	9	882.7**	10	616.6**
CSS vs. FGM × time	5	5.8	3	3.1	5	43.0
LFC vs. CSMW × time	5	462.5**	3	347.9**		...
Mon. vs. Cond. × time	5	460.3**	3	531.6**	5	573.6**
Error (time)	60	74.1	60	307.3	45	854.2

[x] Repeated measures analysis of variance (ANOVA) conducted as a split-plot design with soil type as the main factor and time as the split factor. Factor and interaction sums of squares were partitioned into single degree of freedom orthogonal contrasts. Soil type was divided into CSS (suppressive) versus FGM (nonsuppressive) monoculture soils, LFC (conducive) versus CSMW (microwave-treated suppressive) soils, and monoculture (CSS, FGM) versus conducive (LFC, CSMW) soil comparisons. Time in days was partitioned into linear, quadratic, cubic, quartic, and residual polynomial trend contasts. Interaction sums of squares were further partitioned into their individual polynomial trend contasts (results not shown).

[y] Soil moisture was held at a constant matric potential of −100 or −10 J/kg soil, or was under a fluctuating moisture regime of wetting and drying cycles consisting of wetting to saturation followed by a 2-wk drying period.

[z] Sum of squares followed by * or ** indicates a significant effect due to the source of variation, with F significant at $P < 0.05$ and $P < 0.01$, respectively. Variation in degrees of freedom (df) reflects differences in replication numbers or sampling dates among tests. The −100 J/kg test consisted of four replications of four soil types sampled on six dates over time, whereas the −10 J/kg test consisted of six replications and four sampling dates, and the wet/dry test consisted of four replications, three soil types (FGM not used), and six sampling dates.

for qualitative factors and polynomial trend contrasts for quantitative factors. Mean separation for some experiments with qualitative treatments was accomplished using Duncan's multiple range test. Pathogen population dynamics over time were analyzed by repeated measures ANOVA using a split-plot design and further partitioned into planned orthogonal contrasts. Correlation or regression analyses were conducted where appropriate. Residual error terms were plotted and tested for normality. All data expressed as percentages were arcsine-transformed ($\sin^{-1}\sqrt{x}$) before analysis.

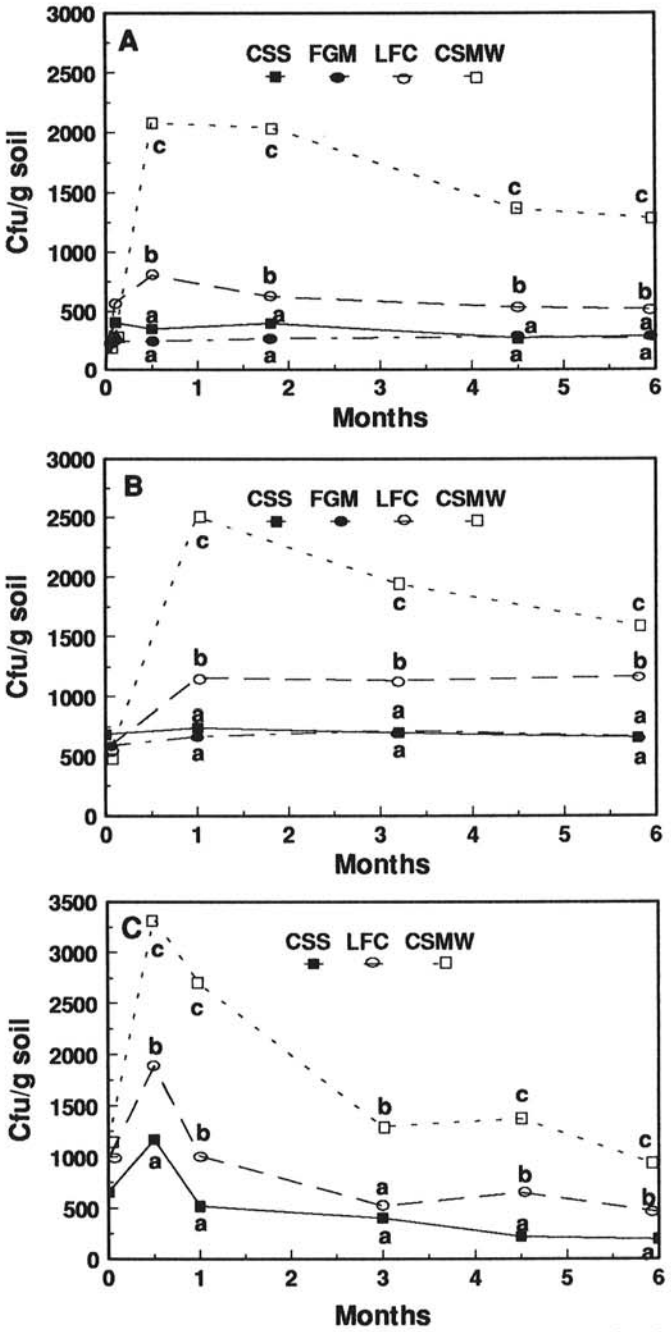

Fig. 1. Populations of *Fusarium oxysporum* f. sp. *niveum* over time in four soils under different moisture regimes (CSS = suppressive, monoculture soil; FGM = nonsuppressive, monoculture soil; LFC = fallow, conducive soil; and CSMW = suppressive soil rendered conducive by microwave treatment). **A,** Constant soil water matric potential of −100 J/kg. **B,** Constant matric potential of −10 J/kg. **C,** Fluctuating moisture potential caused by alternating wetting and drying cycles (soils saturated and allowed to dry in 2-wk cycles). *F. o. niveum* was added to soil as an orange-colored mutant isolate. Values within tests at each sampling date denoted by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). All results are from a single representative experiment.

RESULTS

Production and characterization of orange mutant pathogen strains. A total of eight orange-colored mutant isolates were successfully recovered from parent isolate FG85-1 after repeated screenings with ultraviolet light. Mutation rate for the recovery of orange mutants was in the range of 10^{-4}–10^{-5}. The eight OM isolates varied considerably regarding the characteristics measured, with some isolates revealing significantly impaired capabilities (reductions of 22–89%) relative to the wild-type parent in radial growth, root colonization, and pathogenicity (24). Isolates FG-OR3, FG-OR6, and FG-OR8, however, equaled or surpassed FG85-1 for all characteristics measured in initial tests and were found to be indistinguishable from the wild-type in further evaluations for root colonization and pathogenicity (Table 1). On the basis of these tests, it was concluded that these isolates could be used to adequately represent *F. o. niveum* in field soils and greenhouse tests. The orange pigment was found to be a stable and reliable marker throughout all phases of this research. Isolate FG-OR3 or FG-OR8 was used in all subsequent experiments involving an OM pathogen.

Survival and population dynamics of *F. oxysporum* in field soils. Differences in the population dynamics of *F. o. niveum* were observed among the soils over time (significant soil × time interaction) regardless of soil moisture conditions or initial pathogen inoculum densities (Table 2 and Fig. 1). At constant matric

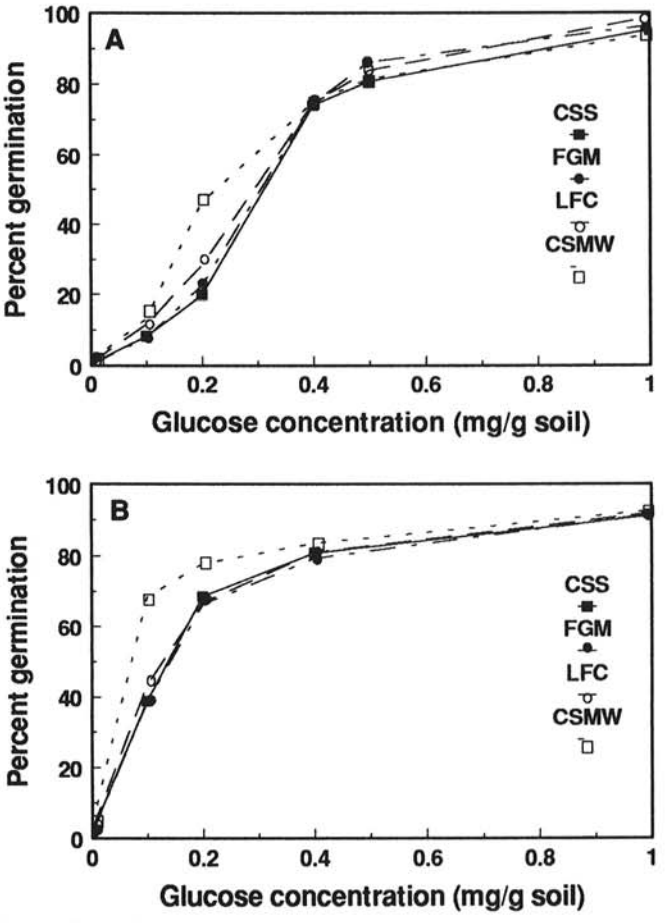

Fig. 2. Chlamydospore germination of two different isolates of *Fusarium oxysporum* f. sp. *niveum* in four soils after additions of glucose (CSS = suppressive, monoculture soil; FGM = nonsuppressive, monoculture soil; LFC = fallow, conducive soil; and CSMW = suppressive soil rendered conducive by microwave treatment). **A,** Race 1 isolate FG85-1 (average of four tests). **B,** Race 2 isolate CS85-4 (average of three tests). Two-way analysis of variance (ANOVA) indicated significant effects due to soil type and glucose concentration (significant linear, quadratic, and cubic trends) for both isolates. Soil × glucose interaction was not significant for either isolate. Three-way ANOVA (soil type, cultivar, and isolate) indicated a significant glucose × isolate interaction, but no other interaction terms were significant.

potentials of −100 and −10 J/kg, populations remained stable at or near initial inoculum densities (200–600 cfu/g soil) in both the suppressive (CSS) and nonsuppressive (FGM) monoculture soils throughout the 6-mo study period. In the fallow, conducive (LFC) soil, populations increased somewhat within the first few weeks, then remained stable throughout the remainder of the experiment. In the microwave-treated soil (CSMW), there was a dramatic initial increase in propagule numbers followed by a slow decline. Partitioning the soil × time interaction into its subsidiary contrasts revealed no differences between CSS and FGM soils over time, whereas differences were evident (further partitioned into significant linear, quadratic, cubic, and quartic trends) between LFC and CSMW soils and between the monoculture soils versus the conducive soils over time (Table 2). Because soil by time interaction was significant, analyses also were conducted among soils within each sampling date (Fig. 1). Pathogen populations were higher in CSMW soil than in other soils throughout the study. Populations in the monoculture soils also were lower than in LFC soil at most sampling dates. In soils under a fluctuating moisture regime, represented by alternating wetting and drying cycles, an initial increase in propagules was generally followed by a gradual decline throughout the 6 mo in all soils. As with the soils with constant moisture potential, the microwave-treated soil had the greatest increase in propagules, and the populations remained the highest throughout the study period.

Chlamydospore germination. There were no differences observed among soils for chlamydospore germination with increasing glucose concentration (0–1.0 mg/g soil) (soil × glucose interaction not significant) for either isolate of *F. o. niveum* tested (Fig. 2). There were significant linear and nonlinear trends due to glucose concentration in all soils for both pathogen isolates. Chlamydospores germinated readily (20–80%) in all soils with small additions of glucose (0.1–0.4 mg/g soil). The only differences among soils were at specific concentrations of 0.1 and 0.2 mg of glucose per gram of soil with isolate CS85-4 and at 0.2 mg/g of soil with isolate FG85-1, where germination was greater in CSMW soil than in any of the field soils. Analyses over all factors indicated there was some variation between the different pathogen isolates tested at the lower glucose levels; isolate CS85-4 germinated more readily than FG85-1 at 0.1 and 0.2 mg of glucose per gram of soil (glucose isolate × interaction term significant), but overall effects were similar for both isolates (soil × isolate and soil × glucose × isolate interaction terms were not significant). Observational data did not indicate any differences or large variation in germ tube lengths or in the lysis of hyphae among the three field soils at any glucose level.

Root colonization by *F. oxysporum*. Surface colonization of Crimson Sweet and Florida Giant watermelon roots by added *F. o. niveum* (OM pathogen) was similar in CSS, FGM, and CSMW soil, whereas colonization was greater in LFC soil than in the other three soils (Table 3). Colonization by indigenous *F. oxysporum* was similar in CSS, FGM, and LFC soils. There was virtually no colonization in CSMW soil due to the elimination of *F. oxysporum* by microwave treatment. There was no significant cultivar effect or interaction with soil type for colonization by the OM pathogen or indigenous *F. oxysporum*, with similar colonization observed on Florida Giant and Crimson Sweet. Colonization followed no discernible pattern of spatial arrangement on the roots. Colonization by the OM pathogen and other *F. oxysporum* were intermixed somewhat randomly over the surface. There was no indication of preferential colonization of root tips or young roots; colonization occurred uniformly over all root types. The ratio of colonization by *F. o. niveum* to colonization by *F. oxysporum* in CSS soil was not different from that in FGM soil for either Florida Giant or Crimson Sweet, but the CSS soil ratio was less than that for LFC soil for Crimson Sweet (soil × cultivar interaction term was significant). The percentage of wilted plants, however, was lower in CSS soil (6.6%) than all other soils planted to Crimson Sweet (47–58%). When planted to Florida Giant, less wilt occurred in CSS soil than in the conducive soils. Cultivar as well as soil type had a significant effect on level of wilt (soil × cultivar interaction not significant), with soils planted to Crimson Sweet generally producing less wilt than those planted to Florida Giant. Percentage of Fusarium wilt was not consistently correlated to the degree of colonization by the OM pathogen, indigenous *F. oxysporum*, or ratio of OM pathogen/other *F. oxysporum*. Soil populations of the pathogen at the time of root sampling were lower in the monoculture soils than in the conducive soils. A repeat of this test showed similar results, except that colonization by the OM pathogen in CSMW soil was consistently higher. With the exception of CSMW soil, which appeared to have unusually low OM colonization in this test compared with subsequent tests, colonization by *F. o. niveum* was correlated with populations of *F. o. niveum* in the soil (Pearson's correlation coefficient $r = 0.76$ and 0.67 for Crimson Sweet and Florida Giant, respectively). Thus, surface colonization ap-

TABLE 3. Surface colonization of roots of two different watermelon cultivars by *Fusarium oxysporum* in four soils in relation to disease incidence and populations of *Fusarium oxysporum* f. sp. *niveum*

Cultivar and soil type[v]	Colonies per 100 cm of root[w]		Colonization ratio[x]	Wilt (%)	OM population[y] (cfu/g of soil)
	OM pathogen	*F. oxysporum*			
Crimson Sweet					
CSS	6.0 a[z]	27.5 b	0.21 a	6.6 a	44 a
FGM	9.8 a	33.1 b	0.37 ab	51.4 b	50 a
LFC	26.8 b	21.3 b	1.36 b	58.0 b	231 b
CSMW	12.9 a	0.2 a	10.12 c	47.1 b	269 b
Florida Giant					
CSS	8.6 a	24.7 b	0.52 a	31.8 a	62 a
FGM	11.3 a	27.0 b	0.43 a	43.8 ab	38 a
LFC	26.4 b	17.0 b	1.96 a	80.3 c	450 c
CSMW	12.8 a	0.3 a	19.00 b	64.8 bc	238 b

[v] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS = Crimson Sweet suppressive, monoculture soil; FGM = Florida Giant monoculture soil (nonsuppressive); LFC = Leesburg fallow conducive soil; CSMW = microwave-treated, Crimson Sweet soil (conducive).

[w] Colonization of roots by the orange mutant (OM) isolate of *F. o. niveum* and all *F. oxysporum* other than the OM pathogen was determined by washing the roots of 3-wk-old plants and embedding them intact in Komada's (23) medium. Four replications of four to six roots each were used. Root length was estimated by the line-intersect method (51).

[x] The colonization ratio represents the mean of the colonization by the OM pathogen divided by the colonization by other *F. oxysporum* calculated for each sample.

[y] Soil population of the OM pathogen was determined by dilution-plating at the time of root colonization measurements. Initial inoculum was approximately 100 colony-forming units (cfu)/g soil.

[z] Means within columns for each cultivar followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test. Two-way analysis of variance indicated a significant soil type × cultivar interaction for the colonization ratio and OM populations in soil only. There was a significant effect due to soil type for all other variables and a significant cultivar effect on percent Fusarium wilt only. All results are from a single representative experiment.

peared to correspond more closely to soil populations rather than reflecting differential colonization in different soils or cultivars.

Since surface colonization was correlated primarily with soil populations, internal colonization (or its approximation) was measured in subsequent tests by plating surface-disinfested watermelon roots. In one such test (with results representative of other tests), in which initial OM inoculum was 200 cfu/g of soil and Crimson Sweet was planted, colonization by the OM pathogen averaged 0.91, 1.40, and 6.53 colonies per 100 centimeters of root for CSS, FGM, and LFC soils, respectively, whereas the corresponding values for colonization by indigenous *F. oxysporum* were 10.83, 14.92, and 5.23 colonies per 100 centimeters of root. There was no difference (Duncan's multiple range test, $P < 0.05$) between CSS and FGM soils for colonization by *F. o. niveum* and *F. oxysporum*, or in their colonization ratio, although the difference between the LFC soil and the two monoculture soils was significant for all three values.

Soil and root microorganism populations. Soil population estimates of general groups of microorganisms in the four soils before planting showed some overall similarities as well as a few notable differences among the soils (Fig. 3A). Bacterial populations were similar, ranging from 6.90 to 7.15 log cfu/g of soil in the four soils. Estimates of actinomycete populations were significantly greater in the two monoculture soils (6.5 and 6.42 log cfu/g soil for CSS and FGM, respectively) than in the conducive soils (5.44 and 6.09 log cfu/g soil for LFC and CSMW, respectively). Fluorescent pseudomonad populations were higher in CSS than in any other soil. Nonfluorescent pseudomonad populations also were greater in CSS than in FGM and CSMW soils. Fungal populations were highest and showed the greatest diversity in colony morphology in LFC soil than in any of the other soils (5.42 vs. 4.94, 4.75, and 4.48 log cfu/g soil for CSS, FGM, and CSMW soils, respectively). Although actual numbers fluctuated from one test to another, differences among the soil population estimates relative to one another were similar in repeated tests.

Estimates of microorganism populations isolated from watermelon roots also were different among the four soils (Fig. 3B). Root populations of bacteria, actinomycetes, fluorescent pseudomonads, and other pseudomonads were greater in both monoculture soils than in LFC or CSMW soil. Fluorescent pseudomonad populations on roots in CSS soil also were greater than in FGM soil (6.00 log cfu/g root in CSS soil and 5.35 log cfu/g root in FGM soil). Fungal populations were greater on roots in LFC than any other soil. Analysis with soil type and source of isolation as factors indicated that prokaryote populations (bacteria, actinomycetes, and pseudomonads) were consistently greater on roots than in soil, with populations averaging 1–2 orders of magnitude greater per gram of roots than per gram of soil. Differences among the soils regarding prokaryote populations also tended to be greater on roots than in the bulk soil. Pseudomonad populations responded differently to source of isolation among the soils (soil type × source interaction terms were significant for fluorescent and nonfluorescent pseudomonads, whereas all other interaction terms were not significant). Overall fungal populations were comparable both in the soil and on roots, although they averaged slightly lower on roots. All population differences were observed with both Florida Giant and Crimson Sweet cultivars, with only slight variations observed between cultivars.

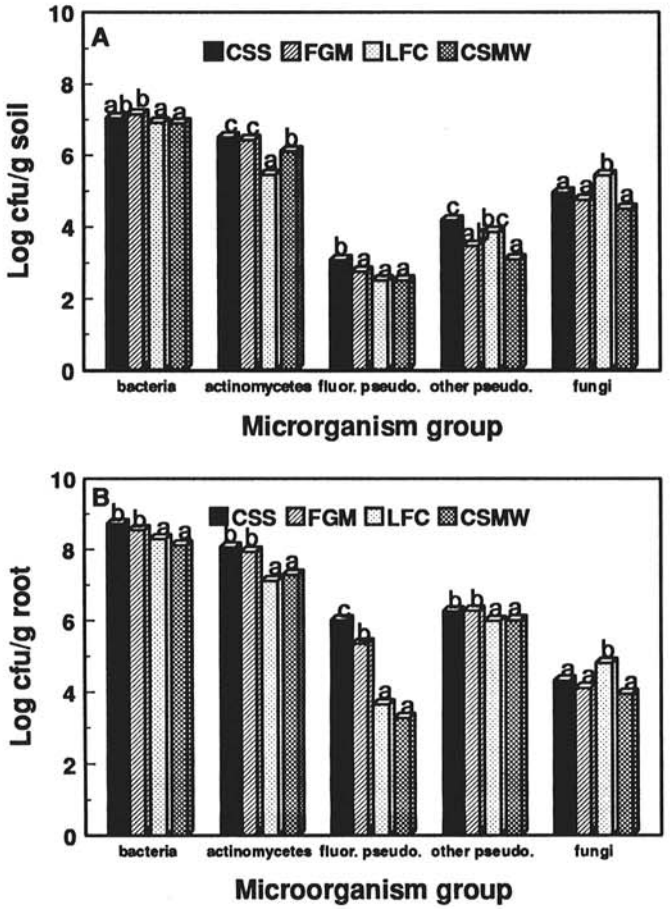

Fig. 3. Population estimates of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in soil and on watermelon roots in four different soils (CSS = suppressive, monoculture soil; FGM = nonsuppressive, monoculture soil; LFC = fallow, conducive soil; CSMW = suppressive soil rendered conducive by microwave treatment). **A,** Soil microorganism populations before planting to watermelon. **B,** microorganism populations on 3-wk-old watermelon roots (combined values for cultivars Florida Giant and Crimson Sweet). Values within each microorganism group topped by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test. Two-way analysis of variance indicated a significant soil × source of isolation interaction for populations of fluorescent and nonfluorescent pseudomonads only. There were significant effects due to soil type for all other variables and due to source of isolation for all other variables except fungi. All results are from a single representative experiment.

Selective elimination of microorganisms by microwave irradiation. Incidence of Fusarium wilt observed in soils that had been exposed to microwave treatments of 0 s (control) and 30 s/kg soil, followed by infestation of the pathogen, were significantly different among the three soils (Fig. 4). These differences reflected the level of suppressiveness of the respective soils; there was less

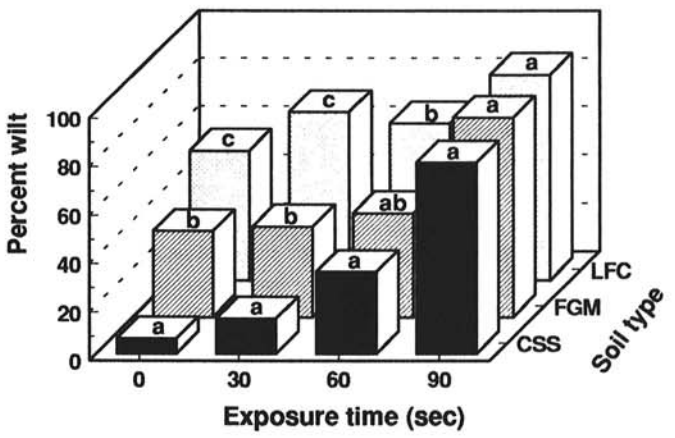

Fig. 4. Fusarium wilt development in three soils exposed to microwave treatments of varying time lengths (CSS = suppressive, monoculture soil; FGM = nonsuppressive, monoculture soil; LFC = fallow, conducive soil). Chlamydospore inoculum of 200 cfu/g of soil was added to each soil after microwave treatment. Values within a given exposure time topped by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test. Two-way analysis of variance indicated a significant soil × exposure time interaction. Partitioning of the interaction sum of squares indicated significant differences between the linear trends for CSS versus FGM and the monoculture soils versus LFC soil.

disease in CSS soil than in FGM and LFC soils, and FGM soil resulted in less wilt than LFC soil. At a microwave exposure of 60 s/kg soil, percent wilt in CSS soil was no longer significantly lower than in FGM soil, demonstrating a reduction in the level of suppression in CSS soil. At 90 s/kg soil, all soils showed high levels of Fusarium wilt, and all differences in suppressiveness among the soils were eliminated. Over time, analysis of microwave exposure indicated that the soils responded differently to microwave exposure (soil × microwave interaction term significant). Further partitioning of the interaction sums of squares into subsidiary orthogonal contrasts revealed significant differences among soils for the linear contrast trend. Regression analysis also indicated differences among the soils with increasing microwave exposure (linear regression analysis, $P < 0.05$, $b = 0.65 \pm 0.11$ and $b = 0.34 \pm 0.11$ for CSS and FGM soils, respectively) with greater change occurring in CSS soil than in FGM or LFC soil.

Microwave irradiation (0–90 s/kg soil), followed by infestation with the OM pathogen, had significant effects on internal root colonization by the OM pathogen, *F. oxysporum*, and other fungi, with significant linear and nonlinear polynomial trends observed for all three (Table 4). Both CSS and FGM soils responded similarly to microwave exposure (soil × microwave interaction term was not significant). Differences between CSS and FGM soils were observed only for colonization by other fungi and for percent wilt, with no differences observed for colonization by the OM pathogen or *F. oxysporum*. Generally, microwave irradiation at exposures of 60 s or less resulted in little change in the internal colonization of roots, but the 90-s treatment allowed drastic increases in pathogen colonization in both CSS and FGM soil. Little to no colonization by *F. oxysporum* or other fungi was observed at the 90-s treatment in either soil. Internal root colonization by *F. oxysporum* was not different between CSS and FGM soils at any level of microwave exposure. Higher levels of colonization by other miscellaneous fungi were observed in FGM than in CSS soil, with significant differences observed at the 0- and 60-s exposure levels. Differences also were observed between CSS and FGM soils for percent wilt at the 0- and 30-s exposure levels, with CSS soil maintaining lower wilt than FGM soils. Percent wilt was positively correlated with colonization by *F. o. niveum* in both soils ($r = 0.80$ and 0.75 for CSS and FGM soils, respectively), and negatively correlated with colonization by indigenous *F. oxysporum* ($r = -0.63$ and -0.62 for CSS and FGM soils, respectively).

Root population estimates of total bacteria, actinomycetes, and fluorescent pseudomonads were higher in CSS soil than in FGM soil over all microwave treatments, whereas root populations of nonfluorescent pseudomonads and fungi were higher in FGM soil than in CSS soil (Table 5). Although microwave exposure also resulted in some effects on root populations, polynomial contrasts indicated no consistent linear or quadratic trend with increasing microwave exposure for bacteria or actinomycete populations. Significant nonlinear trends (quadratic and cubic) were observed for microwave exposure on fluorescent and nonfluorescent pseudomonad populations. Interaction of soil type × microwave exposure was not significant for any microorganism group. The slight effects due to microwave treatment observed on prokaryote populations in this test, including increases in the population of other pseudomonads in both soils at the 90-s treatment, were not observed in a repeat of this experiment, in which total root bacterial populations were relatively unaffected by microwave treatment. Comparison between the soils at each exposure level revealed significantly higher populations of bacteria and fluorescent pseudomonads in CSS soil than FGM soil at the 30- and 90-s exposure levels. Root populations of fungi in FGM soil were significantly greater than in CSS soil at the 60- and 90-s exposures. Populations of nonfluorescent pseudomonads were greater in FGM soil than in CSS soil in the control (0-s exposure). Overall, similar population levels were observed in a repeat of this experiment, although differences between CSS and FGM were not always significant, and microwave treatments generally did not affect prokaryote populations. Combined data from both tests verified the differences in populations of bacteria and fluorescent pseudomonads described here, but actinomycete populations were not different between the two soils. In another test that looked at rhizoplane-only organisms, rhizoplane populations of bacteria and fluorescent pseudomonads were higher in CSS soil than in FGM soil over all microwave treatments.

TABLE 4. Internal colonization of cultivar Crimson Sweet watermelon roots by *Fusarium oxysporum* in two soils exposed to microwave treatments of varying duration

Soil type[u]	Microwave exposure[v] (s/kg of soil)	Colonies per 100 cm of root: OM pathogen[w]	*F. oxysporum*[x]	Other fungi[y]	Wilt (%)
CSS	0	1.02[z]	13.94	1.37	6.2
FGM	0	0.55	14.34	5.77*	35.5*
CSS	30	0.80	7.72	1.66	14.5
FGM	30	2.24	15.51	4.11	37.5*
CSS	60	0.71	5.15	3.26	33.7
FGM	60	0.34	8.11	11.11*	43.0
CSS	90	7.48	0.00	0.63	78.7
FGM	90	14.19	0.00	0.00	82.5

[u] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS = Crimson Sweet suppressive, monoculture soil; FGM = Florida Giant monoculture soil (nonsuppressive).

[v] Duration of microwave exposure in s/kg of soil (2,450 MHz, 700 W) at −10 J/kg matric potential; following microwave treatment all soils were infested with the OM pathogen at 200 cfu/g soil.

[w] Colonization of roots by an orange mutant (OM) isolate of *F. o. niveum* (FGOR-3) was determined on the roots of 3-wk-old plants that had been washed, surface-sterilized in 0.5% sodium hypochlorite for 1 min, rinsed, and embedded intact in Komada's (23) medium. Four replications of four to six roots each were used. Root length was estimated by the line intersect method (51).

[x] All *F. oxysporum* isolates other than those of the OM pathogen were counted; roots were prepared as described above.

[y] All other fungi that were capable of restricted growth on Komada's (23) medium were counted.

[z] Means followed by an asterisk were significantly greater ($P < 0.05$) than the corresponding mean in the other soil type at that exposure level according to Fisher's least significant difference. Linear regression analysis of wilt data ($P < 0.05$) also demonstrated a difference between the two soils with increasing microwave exposure ($b = 0.65 \pm 0.11$ for CSS soil and $b = 0.34 \pm 0.11$ for FGM soil). Two-way analysis of variance indicated significant effects due to soil type for colonization by other fungi and percent wilt only. Soil × exposure time interaction was not significant for any variable. Partitioning of exposure time into linear, quadratic, and residual polynomial trend contasts showed significant linear and/or quadratic trends for all variables. All results are from a single representative experiment.

DISCUSSION

Suppressive soils can be categorized as pathogen suppressive or disease suppressive on the basis of whether they act directly on the pathogen to reduce its population in the soil or act indirectly by reducing the disease-causing activity of the pathogen (6,20,44). However, this distinction may not always be known or a soil may incorporate both means of suppression. Components in the soil may suppress the pathogen directly by destruction of propagules or hyphae through lysis or predation, by inhibiting propagule germination, or by reducing saprophytic growth in some other way (6,40,44). Suppression of the disease-causing activity of the pathogen may involve reduction in parasitic colonization or infection of the host or reduced ability to induce disease after infection (6).

In this study, there was no evidence of active destruction of pathogen propagules in the suppressive soil. Populations of *F. o. niveum* were stable over a 6-mo period, with no substantial decrease in propagules over time. While the pathogen did multiply somewhat in the conducive soils, it did not multiply in the monoculture soils. This indicates some differences in fungistasis among the soils, since the ability of an organism to multiply in a soil is one of the criteria used to determine the presence or absence as well as level of microbiostasis (16).

Chlamydospore germination has been used as an indicator of the fungistatic activity and suppressiveness of soils (6,21,22). No differences were observed in pathogen chlamydospore germination as a response to glucose amendments among the three field soils used in this study. There were no differences noted in germ tube lengths or in the lysis of hyphae. Thus, there was no indication of suppression or reduction of pathogen saprophytic growth within the suppressive soil by these measures. Higher germination was noted in CSMW soil at some glucose levels, although germination was not different overall. However, this soil had its microbiology, nutritional status, and chemistry altered by microwave treatment, and would be expected to have a low level of fungistasis.

Thus, no relationship was observed between suppressiveness and chlamydospore germination in these soils. This differs distinctly from most other Fusarium wilt-suppressive soils that have been studied, in which suppressiveness has been consistently associated with a high level of fungistasis, reduction of saprophytic growth, and the inhibition of chlamydospore germination (2,4,6, 30,40,45). Compared with chlamydospore germination studies in other Fusarium wilt-suppressive soils, CSS soil required much lower levels of glucose to overcome fungistasis and initiate germination. At 0.4 mg of glucose per gram of soil, chlamydospore germination in CSS soil was around 75–80%, whereas in several other reported suppressive soils, chlamydospore germination averaged only 10–35% at similar glucose levels (3,5,22,46,49). In these other studies, germination rates in conducive soils averaged from 60–90% at approximately 0.5 mg of glucose per gram of soil, indicating that fungistasis levels in soils in this study (CSS, FGM, and LFC) are uniformly low and comparable to conducive soils in other studies. Since suppression related to inhibition of chlamydospore germination is usually attributed to a general nutrient competition due to a large antagonistic microbial biomass, it appears that this general suppression evident in other suppressive soils is probably not a major factor in the CSS suppressive soil. However, since the suppressive soil did maintain lower pathogen populations and the pathogen was unable to multiply as readily as in conducive soils, saprophytic growth and development may be affected at some stage other than chlamydospore germination.

High populations of nonpathogenic *F. oxysporum* have been shown to be involved in suppressiveness in some Fusarium wilt-suppressive soils. Alabouvette and others (2,4,30) determined that the primary cause of suppression in the Chateaurenard region soils is a result of intrageneric competition in the immediate vicinity of the roots during the saprophytic development that precedes establishment of *F. oxysporum* at the root surface. Schneider (41), who associated nonpathogenic *F. oxysporum* with suppression of celery wilt in a California soil, attributed the mechanism of suppression to parasitic competition for infection sites on the root. Thus, he distinguished the saprophytic competition that precedes root colonization from parasitic competition for root infection. Tamietti and Pramotton (50) attributed the suppression of flax wilt in three soils in Italy to nonpathogenic strains of *F. oxysporum* by a similar mechanism. Paulitz and co-workers (34,35) introduced nonpathogenic isolates of *F. oxysporum* which reduced Fusarium wilt of cucumber in field soils. Mandeel and Baker (31) later concluded that these isolates incorporated multiple suppression mechanisms, including saprophytic and parasitic competition as well as induction of enhanced

TABLE 5. Microorganism populations on cultivar Crimson Sweet watermelon roots in two soils exposed to microwave treatments of varying duration

Soil type[w]	Microwave exposure[x] (s/kg soil)	Log cfu/g root[y]				
		Bacteria	Actinomycetes	Fluorescent pseudomonads	Other pseudomonads	Fungi
CSS	0	8.03[z]	7.25	5.51	5.68	4.40
FGM	0	7.68	7.10	5.18	6.23*	4.55
CSS	30	8.48*	7.58*	5.54*	6.07	4.26
FGM	30	8.03	7.17	5.04	6.33	5.10
CSS	60	7.87	7.29	5.19	5.70	4.01
FGM	60	7.78	7.10	4.51	6.22	5.02*
CSS	90	8.17*	7.33	6.24*	6.64	3.66
FGM	90	7.86	7.10	5.16	6.64	4.74*

[w] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS = Crimson Sweet suppressive, monoculture soil; FGM = Florida Giant monoculture soil (nonsuppressive).

[x] Values represent the duration of microwave exposure (s/kg soil) (2,450 MHz, 700 W) at −10 J/kg matric potential. After microwave treatment all soils were infested with OM pathogen at 200 cfu/g soil.

[y] Estimates of microorganism populations were made by sonication of roots from 3-wk-old plants in sterile water for 5 min and dilution-plating the resulting suspensions on various agar media. Four replications of two roots each were used. Bacterial populations were estimated on nutrient agar and 1:10 strength tryptic soy agar; actinomycete populations were estimated on alkaline water agar; and pseudomonad populations were estimated on a selective King's medium B (37). Fluorescent pseudomonads were identified by the production of diffusible fluorescent pigment when plates were examined under UV light. Fungal populations were estimated on potato-dextose agar with tergitol and chlortetracycline added.

[z] Means followed by an asterisk were significantly greater ($P = 0.05$) than the corresponding mean in the other soil type at that exposure level according to Fisher's least significant difference test. Linear regression analysis over microwave exposure was not significant ($P < 0.05$) except for populations of fluorescent pseudomonads, other pseudomonads, and fungi in CSS soil ($b = 0.007 \pm 0.002$, 0.007 ± 0.002, and -0.0065 ± 0.0025, respectively) and fluorescent pseudomonads in FGM soil ($b = 0.016 \pm 0.003$). Two-way analysis of variance indicated significant effects due to soil type for all microorganism groups and significant effects due to microwave exposure for populations of fluorescent and nonfluorescent pseudomonads only. Soil × exposure time interaction was not significant for any microorganism group. All results are from a single representative experiment.

resistance in the host.

In this study, surface colonization and internal colonization of watermelon roots by *F. oxysporum* were analyzed so that the possibility of both saprophytic and parasitic competition could be evaluated in this suppressive soil. Overall, colonization levels were similar to those reported for *F. oxysporum* by others (13,14). However, neither surface nor internal colonization measurements revealed significant differences between CSS and FGM soils, although there were differences between the two monoculture soils and the two conducive soils. Surface colonization levels appeared to be affected primarily by soil population levels. Internal colonization by *F. o. niveum* averaged consistently lower in CSS soil, but was not significantly different from colonization in FGM soil. Differences in wilt among the soils did not appear to be related to the levels of colonization by the OM pathogen, indigenous *F. oxysporum*, or the ratio of OM pathogen/other *F. oxysporum*. Thus, saprophytic competition provided by large populations of nonpathogenic *F. oxysporum*, as observed by Alabouvette (2), is apparently not the primary mechanism responsible for suppression in this soil.

Although colonization by the general population of *F. oxysporum* was not related to suppression, it could be that specific isolates of *F. oxysporum* rather than general population levels may be responsible. Schneider (41) observed that many isolates of *F. oxysporum* were capable of infecting roots, but only some of these were effective antagonists. This may explain why the level of colonization by *F. oxysporum* is comparable in CSS and FGM soils, yet only CSS is highly suppressive. The suppressive soil may contain more isolates capable of reducing disease, which are enhanced by the cultivar Crimson Sweet. These isolates may not colonize roots more effectively than others, but may be more effective in suppressing disease.

Indigenous isolates of *F. o. niveum*, which were not marked with the orange phenotype, may be present in the field soils used in these experiments. Since these would be measured as indigenous *F. oxysporum*, they must be taken into consideration, particularly if the numbers of indigenous pathogens differ in CSS and FGM soils. Wilt of susceptible cultivars in unamended CSS soil is consistently low (0–20%), whereas it can be quite variable in FGM soil, ranging from 10–70% wilt and averaging about 30–50% wilt in greenhouse trials (19). However, disease levels in either soil often do not change substantially with addition of moderate amounts of the pathogen (up to 300 chlamydospores per gram of soil); differences in disease levels between the soils may not necessarily be caused by different pathogen populations. On the basis of a number of pathogenicity tests using limited numbers of isolates of *F. oxysporum* (20–50 per test) taken from the soil and roots, the proportion of indigenous pathogenic isolates of *F. o. niveum* relative to the total population of *F. oxysporum* is estimated to be in the range of 10–30% in CSS soil and about 10–40% in FGM soil, with no significant differences observed between these soils in any test (18,19, R. P. Larkin and D. L. Hopkins, *unpublished data*). Although numerous such tests have been conducted, large variabilities and inconsistencies inherent in these tests (due to the large number of isolates needed, laborious screening processes, and the dynamic nature of soil populations) do not allow very accurate estimation of the proportion of pathogens within the soil populations. Thus, although it is not known precisely what percentage of root colonization by indigenous *F. oxysporum* is actually due to unmarked isolates of the pathogen in these experiments, plating of surface-disinfested stems and roots from diseased plants very rarely revealed systemic infection by non-orange-mutant pathogens. Moreover, wilt in the root colonization experiments resulted from systemic infection by the OM pathogen only, so relationships between colonization and level of wilt were not affected. Thus, although it is possible that a slightly larger percentage of colonization by *F. oxysporum* in FGM soil may have been from pathogenic strains other than the OM pathogen, the effect on the overall results appears to be minimal. Nonetheless, the possibility of parasitic competition on the root cannot be fully evaluated without additional experiments that can more accurately monitor colonization by pathogens versus nonpathogens and comparisons of specific antagonist strains.

Population estimates of total bacteria, actinomycetes, fluorescent pseudomonads, and fungi in the soil and on root surfaces were similar to those reported by others using similar soils and methodology (10,43). Populations of these microorganism groups showed significant differences among the different soils, with fluorescent pseudomonad populations on watermelon roots and in the soil being consistently greater in CSS soil than in all others. Crimson Sweet suppressive soil also supported significantly greater bacteria and actinomycete root populations than did the other soils in some tests. Additional related experiments also indicated generally higher populations of bacteria, actinomycetes, and fluorescent pseudomonads on roots in CSS soil than the other soils (24). The substantially higher microorganism populations found on roots as opposed to within the soil in all soils demonstrate the importance of the root on soil microorganisms (12). The greater effect of the root on bacterial populations over fungi is a result of the greater ability of bacteria to quickly colonize roots and most effectively utilize root exudates. Fluorescent pseudomonad populations showed the largest increases throughout this study, both due to soil differences and root influence. Fluorescent pseudomonads are known to be very effective and competitive root colonizers and have often been associated with disease suppression and promotion of plant growth (42). The differences in fluorescent pseudomonad populations between CSS and FGM soils suggest that fluorescent pseudomonads may be responding to differences in root exudates between cultivars, and thus may be important in the suppressive response.

Exposure of the soils to microwave treatments had little effect on overall microorganism numbers on watermelon roots, and there was also no difference in the relative effect between CSS and FGM soils. However, these experiments did demonstrate that at the point at which populations of indigenous *F. oxysporum* were eliminated (90 s), suppression was lost. Disease suppression was also correlated with colonization by *F. oxysporum* in these tests; when populations of *F. oxysporum* were reduced by microwave treatment, wilt increased. At the same time, microwave exposure had no significant linear effect on overall numbers of bacteria, actinomycetes, or pseudomonads on watermelon roots. Overall fungal populations were also not as severely affected as *F. oxysporum* by microwave treatment. However, although overall numbers may not have been affected, species composition and diversity, which were not monitored in this study, may have been altered by microwave exposure. Microwave treatment is known to have a greater effect on fungal populations than on bacterial populations and is considered more desirable than autoclaving or fumigation for eliminating fungal pathogens because it is less disruptive and leaves a large, relatively diverse bacterial biomass (11). This is indirect evidence that *F. oxysporum* or some other fungi, rather than bacteria, may be important in the suppressive response.

The suppressive soil used in this study had already been shown to be different from the majority of the described Fusarium wilt-suppressive soils in many ways (18). In the current study, many additional characteristics of the ecology of the pathogen in this soil have been analyzed, indicating additional differences from other Fusarium wilt-suppressive soils, as well as possible interactions and mechanisms important in the suppressive response. This soil appears to be disease-suppressive, rather than pathogen-suppressive, in that the disease-causing activity of infection and development of disease is where suppression most probably occurs. From this work, the organisms showing the largest differences between suppressive and conducive soils, and thus the most important organisms to study further for their interactions with the pathogen, were indigenous *F. oxysporum* and fluorescent pseudomonads. These two groups also have been most often associated with antagonism or suppression of Fusarium wilt in other soils (2,4,6,21,30,38,39). However, other organisms not yet identified from the general microorganism groups monitored in this study also may be important in suppression.

This study has dealt with general ecological characteristics and

general soil and root populations of microorganisms. Cultivation to watermelon appeared to have some general effects on the microorganism populations in these soils. Further experiments to analyze and evaluate the effects of cultivation of watermelon on microorganism populations have been conducted and are presented and discussed elsewhere (27). The key to understanding this suppression may be in specific antagonistic isolates, which are different among the soils. Cultivar Crimson Sweet may promote specific strains with characteristics which make them more effective as antagonists and are present in greater numbers in CSS soil. The next step in this research will be to look at specific antagonists and their interactions with the pathogen.

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