Colonization of Muskmelon and Nonsusceptible Crops by *Fusarium oxysporum* f. sp. *melonis* and Other Species of *Fusarium*

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ABSTRACT

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The ability of *Fusarium oxysporum* f. sp. *melonis* to colonize roots of a susceptible muskmelon cultivar and six crop species rotated with melons was quantified in the greenhouse and field. Root colonization by *F. solani*, *F. equiseti*, and nonpathogenic strains of *F. oxysporum* also was recorded. Roots of alfalfa, cotton, Honey Dew muskmelon, sugar beet, tomato, and wheat were colonized by *F. o. melonis*. Crops with comparatively low populations of *F. o. melonis* on their roots also tended to have low populations of other *Fusarium* spp. The relative abundance of the four fungi was similar on all seven crops; nonpathogenic strains of *F. oxysporum* were isolated most frequently, followed by *F. o. melonis*. *F. equiseti* was the least frequently isolated of the four fungi in greenhouse experiments, whereas *F. solani* was the least frequently isolated in the field. Differences in colonization frequencies among these fungi were not proportional to differences in their respective population densities in soil. Nonpathogenic strains of *F. oxysporum* had the highest colonization frequency per colony-forming unit per gram of soil.

Additional keywords: Fusarium wilt, soilborne pathogen.

Fusarium oxysporum Schlecht. f. sp. *melonis* Leach & Currence emend. Snyd. & Hans., cause of Fusarium wilt of muskmelon (*Cucumis melo* L.), has been reported to colonize roots of corn and soybean grown in rotation with muskmelon (2). Colonization of rotation crops also has been reported for other formae speciales of *F. oxysporum* (1,4,10,13,17,22).

Root colonization of a nonsusceptible plant by a pathogenic strain of *F. oxysporum* implies the ability to compete with other root-colonizing fungi. Nevertheless, it has been suggested that formae speciales of *F. oxysporum* are not well adapted to compete with the less specialized fungi commonly found in agricultural soils (5). Persistence of wilt pathogens is attributed to the production of chlamydospores, rather than the pathogen's ability to compete with nonpathogenic fungi (5).

One measure of a pathogen's ability to compete with other root-colonizing fungi is relative infection frequency on nonsusceptible crops. Infection frequency commonly is estimated from colonization frequency, that is, the number of sites on a given length of root that produce discrete colonies on an isolation medium (9,11). Infection frequency can be determined for *F. oxysporum* if the pathogen can be distinguished from nonpathogenic strains. Smith and Snyder (22) reported that *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. & Hans. was morphologically distinct from nonpathogenic strains of *F. oxysporum* isolated from field soil from the San Joaquin Valley of California. Similarly, we can distinguish *F. o. melonis* from nonpathogenic strains of *F. oxysporum* isolated from melon field soils in the San Joaquin Valley.

Fusarium wilt is a serious disease in major melon-growing areas in the San Joaquin Valley (8), and there are no previous reports concerning the ability of the pathogen to colonize rotational crops grown in this area. This study was undertaken to determine the relative infection frequency of race 2 of *F. o. melonis*, *F. equiseti* (Corda) Sacc., *F. solani* (Mart.) Sacc., and nonpathogenic strains of *F. oxysporum* on roots of six crop plants commonly grown in rotation with susceptible muskmelon cultivars. A preliminary account of this work has been published (6).

MATERIALS AND METHODS

Greenhouse studies in naturally infested soil. Colonization by *F. o. melonis* was quantified on roots of wheat (*Triticum aestivum* L. 'Yecora Rojo'), alfalfa (*Medicago sativa* L. 'Moapa 69'), sugar beet (*Beta vulgaris* L. 'SS-Y1'), cotton (*Gossypium hirsutum* L. 'SJ-2'), tomato (*Lycopersicon esculentum* Mill. 'UC 82'), muskmelon (*C. melo* L. 'Greenflesh Honey Dew'), and a western shipping-type muskmelon (*C. melo* L. 'PMR 45-SJ') that is susceptible to Fusarium wilt. Greenflesh Honey Dew is susceptible in a root-dip assay (26) but does not develop Fusarium wilt in the field. A Panhill clay loam soil, obtained from a commercial melon field naturally infested with race 2 of *F. o. melonis*, was combined with sterile coarse sand (3:1, v/v) and blended in a motorized mixing drum. Bulk density of the soil-sand mix was approximately 1.2 times greater than the unamended soil. Each crop was seeded in a pot containing approximately 300 g of soil-sand mix. The experiment included three replicates in a completely randomized design. Each pot was kept in a clay saucer, which was filled with water whenever the soil surface became dry. Temperatures were 28 ± 8 C during the day and 18 ± 3 C at night.

Root samples were removed from each pot along with most of the soil 4 wk after seeding. Roots in the bottom 2 cm of the pot were removed and not included in the sample assayed. Loosely adhering soil was removed with running tap water. Remaining soil was removed by three 30-min washes in 1% sodium hexametaphosphate, with a tap-water rinse between each wash. After the final washing, roots were stored in sterile distilled water for up to 24 hr at 4 C before they were placed on Komada's selective medium (14) amended with 1 ml/L of tergitol NP-10 (KM). Roots ≤ 1 mm in diameter that were not obviously damaged or discolored were laid in parallel rows on the agar surface in 9-cm-diameter plastic petri dishes. A minimum of 100 cm of roots was cultured per replicate (50 cm/plate). Plates were incubated at room temperature (22 ± 3 C) with 12 hr of light per day provided by two 34-W fluorescent tubes. Colonies emerging from plated roots were counted 6 or 7 days later. Species of *Fusarium* were identified on the basis of colony morphology on potato-dextrose agar (PDA) and spore morphology on carnation leaf agar (CLA) (18).

Isolations were made from shoot tissue of the sampled plants. Stem sections were taken from the cotyledonary node of all crops, except wheat and sugar beet where shoot tissue was the first stem node above the soil line and petioles of the first and second true leaves, respectively. Tissue pieces were surface disinfested by rinsing with tap water, followed by immersion for 30 sec in 70% ethanol and 90 sec in 0.5% sodium hypochlorite. Treated tissue pieces were placed directly on KM.

Inoculum densities of *F. o. melonis* and other *Fusarium* spp. in the soil-sand mixture were estimated by soil dilution plating (16,25). Air-dried soil was homogenized in a blender, and 5- or 10-g subsamples were suspended in 200 ml of 1% sodium hexametaphosphate and agitated on a shaker for 30 min. The suspension was diluted 1:4 (v/v) in 0.1% water agar, and 1.0-ml samples were spread over the surface of plates containing KM.

Generation and characterization of benomyl-resistant strains. Cornmeal agar (CMA) plates completely colonized by race 2 of *F. o. melonis* were exposed to ultraviolet light (254 nm) for 30 sec at a distance of 6 cm; radiant energy at this distance was approximately 300 mW cm^{-2}. Plates were overlaid with Puhalla's minimal medium (21) amended with benomyl at 5 μg a.i./ml and maintained in darkness for at least 24 hr. After 5 to 10 days at 22 $\pm$ 3 C, colonies on the agar surface were mass transferred to fresh plates of minimal medium amended with benomyl. Transfers of mycelium that continued to grow in the presence of benomyl were mass transferred to water agar without benomyl, and these cultures were hyphal tipped to minimal medium amended with benomyl. Benomyl-resistant strains obtained by this procedure were tested for pathogenicity on susceptible muskmelon cultivar Top Mark in a seedling root-dip assay (12). Benomyl-resistant strains were recovered from diseased seedlings. A single-spore derivative of one of these strains, confirmed to have wild-type morphology and growth rate on CMA, was designated P2/19B and used in subsequent experiments.

Greenhouse studies in artificially infested soil. Benomyl-resistant strain P2/19B of race 2 of *F. o. melonis* was grown on CMA for 15 days at room temperatures under lights as previously described. Spores were washed from colonized CMA plates with sterile water and added to a Yolo loam soil collected near Davis, CA. This soil, which had no detectable indigenous population of *F. o. melonis*, was combined with sterile, coarse sand (3:1, v/v) before inoculum was added. After the spore suspension was added, the soil-sand mixture was air dried and stored at room temperature (23 $\pm$ 3 C); it was assayed periodically over the next 6 wk by soil dilution plating. Inoculum density of the soil-sand mix was adjusted to a final level of approximately 100 colony-forming units (cfu) of *F. o. melonis* per gram soil by adding noninfested soil and sand. The seven crops grown in the naturally infested field soil also were grown in artificially infested soil; temperatures were 32 $\pm$ 11 C during the day and 21 $\pm$ 2 C at night. Root samples were harvested and processed as previously described.

Field studies. To corroborate the results of our greenhouse experiments, a preliminary field test was conducted to determine colonization frequencies of the four *Fusarium* spp. in a commercial field naturally infested with race 2 of *F. o. melonis*. Each crop was seeded in three replicate plots, in a completely randomized design; each replicate consisted of a single row 4.5 m long. Six weeks after seeding, whole plant samples were collected, including as much of the root system as possible. Four randomly selected plants were taken from each replicate, and roots were washed and placed on KM as described for greenhouse-grown plants. Also, stem sections were taken from each plant as described previously, surface disinfested, and placed on KM.

Plant samples also were collected at 10, 12, and 15 wk after planting for all crops except wheat. As before, four plants were taken from each replicate but isolations were made only from the taproots, which were washed in the same manner as the previous root samples. Several cross sections, 2–3 cm long, were taken from each taproot. Sample weights varied with crop, reflecting differences in the mass of their taproots. The smallest samples were taken from alfalfa (2–3 g/sample) and the largest from sugar beet (15–20 g/sample). Tissue pieces were suspended in sterile water and homogenized in a blender for 2–3 min. Dilutions of the resultant homogenate were spread over the surface of KM. For both cotton and tomato, only the cortex, which readily separated from the central stele, was assayed by this procedure. Sections of the stele from cotton and tomato taproots were surface disinfested as previously described and placed directly on KM.

After the first washing of taproot samples, the sodium hexametaphosphate solution was centrifuged at 16,000 *g* for 15 min. The resultant pellet was dried at 35 C for 24 hr. Samples of 5-, 10-, or 25-mg of this material were resuspended in sterile water and spread over the surface of KM.

Isolations also were made from shoot tissue of field-grown plants. Samples were taken near the cotyledonary node, or, in the case of sugar beets, from petioles of lower leaves. Shoot tissue was surface disinfested, as described above, and placed on KM.

Several weed species growing in or near field plots were collected. Roots were washed and isolations were made as described for crop plants. A minimum of 50 cm of roots from each weed species collected was examined for the presence of *F. o. melonis*.

Vegetative compatibility tests. Nitrate nonutilizing (*Nit*) mutants were selected from isolates of *F. oxysporum* by culturing them on a medium containing chlorate as described by Puhalla (21). *Nit* mutants were tested for vegetative compatibility with tester strains of race 2 of *F. o. melonis* (12).

Data analysis. For each experiment, analysis of variance (ANOVA) was used to test for significant differences in colonization of seven crops by *F. o. melonis*. Similar tests were done for *F. equiseti*, *F. solani*, and nonpathogenic *F. oxysporum*. ANOVA also was used to evaluate differences in colonization frequency of four fungi on the same crop. Mean comparisons were made with Duncan's multiple range test. Within each experiment, colonization frequencies averaged over all seven crops and a crop $\times$ fungus interaction were evaluated by a two-way ANOVA (23). When samples were taken from the same plot at different times, data were analyzed by repeated measures ANOVA. ANOVA and mean comparisons were performed on log-transformed data; nontransformed data are reported. Bartlett's test was used to confirm homogeneity of variances. Statistical computations were performed with NCSS versions 5.0 and 5.3 (J. L. Hintze, Kaysville, UT) on an IBM PC-AT.

RESULTS

Identification of *Fusarium* spp. *F. o. melonis*, other isolates of *F. oxysporum*, *F. solani*, and *F. equiseti* were identified by colony morphology on KM. *F. o. melonis* was characterized by dense, whitish aerial mycelium with a reddish-purple pigment that diffused into the agar. Nonpathogenic strains of *F. oxysporum* produced buff-colored colonies with very little mycelium and abundant sporodochia. Differences in colony morphology also were apparent on PDA. On CLA, both *F. o. melonis* and other isolates of *F. oxysporum* produced macroconidia characteristic of the species (18), with no clear distinction between pathogenic and nonpathogenic strains.

To confirm the correlation between colony morphology and pathogenicity, a total of 97 colonies of both morphological types were isolated from roots and tested for virulence on seedlings of susceptible muskmelon cultivar Top Mark in a greenhouse root-dip assay (12). Eighty-one isolates were identified as *F. o. melonis* on the basis of colony morphology, including a minimum of 10 cultures isolated from each of the seven crops studied. All 81 induced symptoms typical of Fusarium wilt, comparable to those on muskmelon seedlings inoculated with strain P2 of race 2 of *F. o. melonis* (12). At least six of the 10 seedlings inoculated with a virulent strain eventually died. Water-treated controls always were undamaged by the procedure. Sixteen colonies identified as nonpathogenic strains of *F. oxysporum* did not induce symptoms on any of the inoculated muskmelon seedlings.

Colonies identified as *F. solani* on root-isolation plates had whitish aerial mycelium, generally quite distinct in texture from colonies of *F. o. melonis*. Agar beneath colonies of *F. solani* either lacked pigmentation or had a beige to brownish color. *F. equiseti* was readily distinguished from all other fungi on KM as compact, beige to orange colonies. This color generally was apparent in both the aerial mycelium and the underside of the colony. Other fungi identified on KM as root colonizers included *Penicillium* spp., which were seen on less than 5% of the isolation plates, and, in order of decreasing frequency, *Trichoderma* spp., *Cylindrocarpon* spp., *F. proliferatum* (Matsushima) Nirenberg, and *Aspergillus* spp.

Colonization in naturally infested soil. Mean inoculum densities in naturally infested soil were calculated from soil dilution plates representing five independent samples. Mean colony-forming units per gram of soil plus or minus the standard error were as follows: *F. o. melonis*, 270 ± 50; *F. equiseti*, 530 ± 100; *F. solani*, 1,000 ± 140; and nonpathogenic *F. oxysporum*, 330 ± 90.

A two-way ANOVA showed significant differences among the four fungi ($P = 0.001$), which accounted for most of the observed variation in root colonization (Table 1). There also was a significant crop effect on colonization ($P = 0.001$), but there was no significant crop × fungus interaction.

Colonization by *F. o. melonis* of Honey Dew muskmelon, sugar beet, cotton, and wheat was statistically equivalent ($P = 0.05$) to colonization of susceptible muskmelon cultivar PMR 45 (Table 1). Colonization of alfalfa and tomato by *F. o. melonis* was significantly less than recorded for muskmelon cultivar PMR 45.

Nonpathogenic strains of *F. oxysporum* also were isolated from roots of all crops, but differences in colonization were not significant ($P = 0.05$) (Table 1). Nonpathogenic strains of *F. oxysporum* were found at levels statistically equivalent ($P = 0.05$) to *F. o. melonis* on roots of every crop except alfalfa, where nonpathogenic strains were more abundant. *F. solani* and *F. equiseti* were isolated at low frequencies from all seven crops. *F. equiseti* was the least prevalent colonizer of roots on all crops, whereas *F. solani* always occurred at frequencies intermediate between *F. equiseti* and *F. o. melonis*. Differences in colonization of the seven crops by either *F. solani* or *F. equiseti* were not significant ($P = 0.05$).

No fungi were isolated from surface-disinfested shoot tissue of any of the sampled plants, including muskmelon cultivar PMR 45, and none of the plants showed any symptoms of Fusarium wilt.

When this experiment was conducted a second time (data not shown), results were similar to those in Table 1. Differences among fungi represented the largest source of variation, whereas differences among crops were significant ($P = 0.05$) but much less important. A three-way ANOVA of data from both experiments indicated weak but significant crop × experiment and fungus × experiment interactions ($P = 0.05$).

Colonization in artificially infested soil. Colony morphology on KM and resistance to benomyl were used to identify *F. o. melonis* (P2/19B) introduced into Yolo loam field soil. No benomyl-resistant isolates of *F. oxysporum* were recovered from Yolo loam soil to which *F. o. melonis* had not been introduced.

Inoculum densities in artificially infested soil were estimated from five independent samples. Mean colonly-forming units per gram of soil plus or minus the standard error were as follows: *F. o. melonis*, 70 ± 25; nonpathogenic *F. oxysporum*, 40 ± 20; *F. equiseti*, 50 ± 20; and *F. solani*, 150 ± 50.

A two-way ANOVA showed significant differences among fungi ($P = 0.001$) and among crops ($P = 0.001$) and a significant crop × fungus interaction ($P = 0.01$) (Table 2). Muskmelon cultivars PMR 45 and Honey Dew were the most frequently colonized by *F. o. melonis*; roots of sugar beet and tomato were the least frequently colonized by *F. o. melonis*. Nonpathogenic *F.*

TABLE 1. Root colonization of seven crops in soil naturally infested with *Fusarium oxysporum* f. sp. *melonis* 4 wk postplanting under greenhouse conditions

Crop	Colonies per 100 cm of root[a]			
	F. o. melonis	*F. oxysporum*[b]	*F. equiseti*	*F. solani*
Tomato (UC 82)	17.8 a BC	35.7 a C	2.0 a A	8.0 a B
Alfalfa (Moapa 69)	22.0 ab C	50.8 a D	5.0 a A	8.7 a B
Cotton (SJ-2)	33.2 bc B	43.5 a B	9.2 a A	12.2 a A
Wheat (Yecora Rojo)	34.0 bc BC	60.2 a C	6.8 a A	18.0 a B
Muskmelon (PMR 45)	40.3 c BC	48.5 a C	6.5 a A	18.8 a B
Muskmelon (Honey Dew)	42.5 c B	46.7 a B	6.8 a A	8.3 a A
Sugar beet (SS-Y1)	48.2 c C	41.3 a C	4.5 a A	16.0 a B

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Observed F[c]
Total	83	13.21	
Fungus	3	10.30	125.76***
Crop	6	0.79	4.84***
Crop × fungus	18	0.59	1.19
Error	56	1.53	

[a] Means of three replicates; each replicate included 100 cm of root. Means in the same column followed by the same lowercase letter and means in the same row followed by the same uppercase letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Population densities in soil at seeding were 270 ± 50, 330 ± 90, 530 ± 100, 1,000 ± 140 colony-forming units/g for *F. o. melonis*, other *F. oxysporum*, *F. equiseti*, and *F. solani*, respectively.
[b] *F. oxysporum* other than *F. o. melonis*.
[c] F values are significant at $P = 0.001$ (***).

TABLE 2. Root colonization of seven crops in field soil artificially infested with a benomyl-resistant strain of *Fusarium oxysporum* f. sp. *melonis*

Crop	Colonies per 100 cm of root[a]			
	F. o. melonis	*F. oxysporum*[b]	*F. equiseti*	*F. solani*
Tomato (UC 82)	0.4 a AB	2.6 a B	0.0 a A	2.8 b B
Sugar beet (SS-Y1)	0.7 a A	2.6 a A	0.0 a A	0.2 a A
Alfalfa (Moapa 69)	3.4 ab B	5.0 ab B	0.6 a A	5.0 b B
Cotton (SJ-2)	10.2 bc B	7.5 b B	1.2 a A	2.9 b AB
Wheat (Yecora Rojo)	12.0 bc B	11.1 b B	0.0 a A	2.7 b AB
Muskmelon (PMR 45)	16.0 c C	5.4 ab BC	0.6 a A	2.2 ab AB
Muskmelon (Honey Dew)	24.0 d B	3.4 ab AB	1.3 a A	8.5 b AB

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Observed F[c]
Total	83	16.41	
Fungus	3	4.98	24.56***
Crop	6	4.86	11.98***
Crop × fungus	18	2.80	3.30**
Error	56	3.78	

[a] Means of three replicates; each replicate included 100 cm of root. Means in the same column followed by the same lowercase letter and means in the same row followed by the same uppercase letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Population densities in soil at seeding were 70 ± 25, 40 ± 20, 50 ± 20, and 150 ± 50 colony-forming units/g for *F. o. melonis*, other *F. oxysporum*, *F. equiseti*, and *F. solani*, respectively.
[b] *F. oxysporum* other than *F. o. melonis*.
[c] F values are significant at $P = 0.001$ (***) or $P = 0.01$ (**).

oxysporum was most frequently isolated from roots of wheat and cotton and least frequently from tomato and sugar beet. *F. equiseti* was not isolated from tomato, sugar beet, or wheat and only infrequently from the other crops. *F. solani* occurred at the highest frequency on roots of Honey Dew muskmelon and alfalfa and at the lowest frequency on sugar beet. No fungi were isolated from shoot tissue of any of the sampled plants and there were no visible indications of damage to any of the plants whose roots were infected with *F. o. melonis*.

When this experiment was conducted a second time, similar results were obtained (data not shown), except that colonization frequencies by *F. o. melonis*, averaged over all crops, were significantly lower than in the first experiment. This may have been a reflection of lower viability of propagules of *F. o. melonis* in the artificially infested soil (30 ± 20 cfu/g) at seeding of the second experiment.

Some differences occurred in the amount of root colonization in naturally infested soil compared with artificially infested field soil, especially for sugar beet, which frequently was colonized by *F. o. melonis* in naturally infested soil and the least frequently colonized by this fungus in artificially infested soil. Consequently, colonization frequencies of sugar-beet roots in artificially and naturally infested soil were compared in a separate experiment. Greenhouse temperatures were comparable to those during the first experiment in artificially infested soil. In this test, no colonies of *F. o. melonis* were recovered from roots in the artificially infested soil; three colonies per 100 cm of root were recovered from plants grown in naturally infested soil.

Colonization of field-grown plants. Soil population densities in the field were estimated from samples taken at five randomly selected locations. Mean colony-forming units per gram of soil plus or minus the standard error were as follows: *F. o. melonis*, 320 ± 70; nonpathogenic *F. oxysporum*, 410 ± 85; *F. equiseti*, 410 ± 95; and *F. solani*, 560 ± 120.

TABLE 3. Root colonization of seven crops by *Fusarium oxysporum* f. sp. *melonis* and other *Fusarium* spp. under field conditions

Crop	Colonies per 100 cm of root length[a]			
	F. o. melonis	*F. oxysporum*[b]	*F. equiseti*	*F. solani*
Sugar beet (SS-Y1)	4.3 a A	15.2 ab B	23.8 a B	2.3 ab A
Wheat (Yecora rojo)	4.5 ab A	10.7 a A	7.3 a A	3.8 abc A
Tomato (UC 82)	4.6 ab B	15.7 ab C	10.5 a C	2.0 a A
Cotton (SJ-2)	8.3 abc A	23.8 bc B	10.7 a AB	6.2 cd A
Alfalfa (Moapa 69)	10.5 bc AB	22.7 bc C	11.7 a B	4.8 bcd A
Muskmelon (Honey Dew)	16.3 c A	23.3 bc A	20.1 a A	10.3 d A
Muskmelon (PMR 45)	18.3 c B	24.8 c B	17.5 a B	10.7 d A

Analysis of Variance

Source of variation	Degrees of freedom	Sums of squares	Observed F^c
Total	83	10.03	
Fungus	3	4.38	44.74***
Crop	6	2.56	13.05***
Crop × fungus	18	1.26	2.15*
Error	56	1.83	

[a] Means of three replicates; each replicate included 200 cm of root. Means in the same column followed by the same lowercase letter and means in the same row followed by the same uppercase letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Population densities in soil at seeding were 320 ± 70, 410 ± 85, 410 ± 95, and 560 ± 120 colony-forming units/g for *F. o. melonis*, other *F. oxysporum*, *F. equiseti*, and *F. solani*, respectively.
[b] *F. oxysporum* other than *F. o. melonis*.
[c] *F* values are significant at $P = 0.001$ (***) or $P = 0.05$ (*).

Colonization of plant roots by these fungi is shown in Table 3. A two-way ANOVA showed significant differences among fungi ($P = 0.001$), a significant effect of crop ($P = 0.001$), and a significant crop × fungus interaction ($P = 0.05$). *F. o. melonis* most frequently colonized roots of muskmelon cultivars PMR 45 and Honey Dew, with significantly lower frequencies on tomato, wheat, and sugar-beet roots ($P = 0.05$). The same pattern of colonization was found for *F. solani* and nonpathogenic strains of *F. oxysporum*. There were no significant differences in the frequency of *F. equiseti* on the different crop plants. *F. equiseti* and nonpathogenic strains of *F. oxysporum* were generally the most abundant of the four fungi on plant roots, whereas *F. solani* was usually the least abundant.

Taproot samples were collected at three different times during the growing season. Analysis by repeated measures ANOVA indicated a significant time × fungus interaction when colonization frequencies were averaged over all crops (analysis not shown). However, when each crop was examined separately, repeated measures ANOVA revealed no significant time × fungus interaction. Similarly, for each fungus there was no significant time × crop interaction. Consequently, mean comparisons were made among fungi on the same crop and among crops colonized by the same fungus, with data averaged over all three sampling dates (Table 4).

The four fungi were recovered from all crops, with two exceptions. *F. equiseti* never was isolated from cotton taproots, and only *F. o. melonis* was isolated from taproots of muskmelon cultivar PMR 45. In the latter case, other fungi may have been present and not detected because of the extremely high concentration of *F. o. melonis* in the taproot homogenates. *F. o. melonis* was recovered at significantly higher levels from muskmelon cultivar PMR 45 than from the other crops. Taproots of muskmelon cultivar Honey Dew supported significantly higher levels of *F. o. melonis* than alfalfa or cotton.

Because the stele was excluded from taproot homogenates of cotton and tomato, individual tissue pieces were surface disinfested and placed on KM. *F. o. melonis*, *F. equiseti*, *F. solani*, and nonpathogenic *F. oxysporum* were all recovered from stele sections taken from taproots of both tomato and cotton. Seven isolates (two from cotton and five from tomato) were judged to be *F. o. melonis* based on their colony morphology on KM and PDA. *Nit* mutants from each of these seven isolates were paired with tester strains of *F. o. melonis* race 2 (12). All pairings resulted in heterokaryon formation, indicating vegetative compatibility with the melon wilt pathogen.

No fungi were isolated from the surface-disinfested shoot tissue of plants collected for taproot samples, with the exception of

TABLE 4. Isolations of *Fusarium* spp. from taproots of field-grown plants

Crop	Colony-forming units per gram fresh weight of root tissue[a]			
	Fusarium oxysporum f. sp. *melonis*	*F. oxysporum*[b]	*F. equiseti*	*F. solani*
Cotton (SJ-2)	3 a A	2 ab A	0 a A	3 a A
Alfalfa (Moapa 69)	3 a A	2 ab A	1 a A	2 a A
Tomato (UC 82)	8 ab A	3 bc A	7 b A	9 a A
Sugar beet (SS-Y1)	16 ab B	14 c B	9 b B	3 a A
Muskmelon (Honey Dew)	46 b A	5 bc A	6 b A	6 a A
Muskmelon (PMR 45)	32,000 c B	0.a A	0 a A	0 a A

[a] Means of data from samples collected at three different times; on each occasion, four plants were sampled from each of three replicates. Means in the same column followed by the same lowercase letter and means in the same row followed by the same uppercase letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] *F. oxysporum* other than *F. o. melonis*.

F. o. melonis, which frequently was isolated from stems of muskmelon cultivar PMR 45. Thus, many symptomless plants apparently were systemically infected by *F. o. melonis*. Fusarium wilt symptoms were evident on muskmelon cultivar PMR 45 in the field, but these plants were not included in any of the samples. None of the other crops showed any symptoms attributable to infection by *F. o. melonis*. A few cotton plants were damaged by Verticillium wilt.

On each of the three dates that taproot samples were collected, population levels were determined for *F. o. melonis*, *F. equiseti*, *F. solani*, and nonpathogenic strains of *F. oxysporum* in rhizosphere soil associated with taproots. Analysis of these data by repeated measures ANOVA indicated time × treatment interactions that precluded averaging data from all three sampling dates for purposes of mean comparisons. Consequently, for comparative purposes, data are presented only for the first sampling date (Table 5). There was a dramatic difference in the number of colonies of *F. o. melonis* associated with muskmelon cultivar PMR 45 relative to the other crops. Only *F. o. melonis* was isolated from rhizosphere soil of muskmelon cultivar PMR 45. *F. o. melonis* and *F. solani* were not recovered from the rhizosphere of cotton and nonpathogenic F. *oxysporum* was not isolated from the rhizosphere of muskmelon cultivar Honey Dew, but low levels of all four fungi were associated with all other crops.

Results from the other two sampling dates (data not shown) were similar to those shown in Table 5, with the following exceptions. Nonpathogenic strains of *F. oxysporum*, not detectable on the first sampling date, were recovered from rhizosphere soil of muskmelon cultivar PMR 45 at 670 cfu/g on the second sampling date. Relatively high levels of *F. equiseti* were recovered from rhizosphere soil associated with Honey Dew muskmelon and tomato on the third sampling date, averaging 4,000 and 320 cfu/g of soil, respectively. *F. equiseti* was not isolated from rhizosphere soil collected from any crop on the second sampling date.

Colonies identified as *F. o. melonis* were isolated from roots of several weed species growing in the experimental field. This included filaree (*Erodium cicutarium* (L.) L'Hér.), tumbleweed (*Amaranthus albus* L.), nightshade (*Solanum nodiflorum* Jacq.), and sowthistle (*Sonchus oleraceus* L.).

Colonization rates. Differences in colonization frequencies among fungi may reflect differences in fungal population densities in soil. For this reason, comparisons among fungi can be facilitated by expressing colonization frequencies per colony-forming units per gram of soil, as described by Huisman (11). Accordingly, for each fungus, colonization frequencies in each replicate of each crop were divided by the mean soil inoculum density of the fungus. These values were averaged over all seven crops to obtain a mean colonization rate, (colonies/100 cm of root)/(cfu/g soil), for each fungus in each experiment (Fig. 1). *F. o. melonis* and nonpathogenic *F. oxysporum* had higher colonization rates than either *F. equiseti* or *F. solani* in both naturally and artificially infested soil under greenhouse conditions. In the field, *F. equiseti* had a colonization rate comparable with *F. o. melonis*, whereas *F. solani* had the lowest rate. Overall, higher colonization rates were observed in the greenhouse than in the field.

TABLE 5. Isolations of *Fusarium* spp. from rhizosphere soil associated with taproots of field-grown plants

Crop	Colony-forming units per gram of soil[a]			
	Fusarium oxysporum f. sp. *melonis*	*F. oxysporum*[b]	*F. equiseti*	*F. solani*
Cotton (SJ-2)	0 a A	56 b B	60 bc B	0 a A
Sugar beet (SS-Y1)	3 ab A	15 ab A	270 c A	5 a A
Alfalfa (Moapa 69)	4 ab A	8 ab A	4 ab A	4 a A
Tomato (UC 82)	6 ab A	7 ab A	40 abc A	7 a A
Muskmelon (Honey Dew)	140 b A	0 a A	80 bc A	50 a A
Muskmelon (PMR 45)	36,800 c A	0 a B	0 a B	0 a B

[a] Soil recovered from the first sodium hexametaphosphate wash of taproots collected from 15-wk-old plants. Each entry represents the mean of three replicates.
[b] *F. oxysporum* other than *F. o. melonis*.

DISCUSSION

Ranking of the four fungi by colonization frequency was similar on all crops tested. For example, nonpathogenic strains of *F. oxysporum* were nearly always the most frequently isolated, whereas *F. equiseti* and *F. solani* were much less frequently isolated. Such differences among fungi always represented the largest source of variation in root colonization. Differences among crops, though significant, were less important. The crop × fungus interaction was significant in some experiments but it was always the smallest source of variation.

The limited importance of a crop × fungus interaction also was indicated by correlation analysis of field data, which showed that the number of colonies of *F. o. melonis* was positively correlated with the total number of colonies of the other three *Fusarium* spp. on roots of the same crop ($r = 0.64$; $P = 0.001$). This suggests that differences among crops reflected the suitability of roots for colonization by *Fusarium* spp. in general, rather than a specific interaction with *F. o. melonis*. Huisman (11) has reported a similar lack of specificity for *Verticillium dahliae* Kleb.

Differences in colonization rates suggest that, of the four fungi studied, nonpathogenic strains of *F. oxysporum* are the most efficient colonizers of plant roots (Fig. 1). This is consistent with other reports showing *F. oxysporum* to be an important primary colonizer of plant roots (19,22,24). *F. o. melonis* had a colonization rate comparable to the nonpathogenic strains only in the artificially infested soil. This may reflect a more favorable nutritional status of propagules produced axenically on a comparatively rich medium (CMA) relative to those indigenous to soil (20).

Colonization rates reported here are influenced by errors in estimates of population densities in soil. However, because soilborne *Fusarium* spp. generally persist in soil as chlamydospores (3,15,25), soil dilution plating should provide a reasonable measure of the population densities. For example, Nash and Snyder (16) found this method reliable for estimating the population density of *F. solani* f. sp. *phaseoli* (Burk.) Snyd. & Hans. in field soil. Moreover, relative differences in colonization

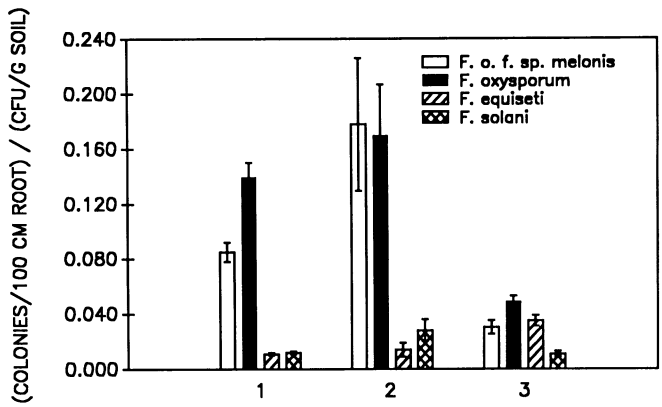

Fig. 1. Colonization rates (colonies, 100 cm^{-1} root, colony-forming units [cfu] g^{-1} soil) of roots by *Fusarium oxysporum* f. sp. *melonis*, *F. equiseti*, *F. solani*, and nonpathogenic *F. oxysporum* in: **1,** naturally infested field soil under greenhouse conditions, **2,** field soil artificially infested with *F. o. melonis* and naturally infested with the other three fungi under greenhouse conditions, and **3,** under field conditions. Each bar represents the average colonization rate for seven crops (alfalfa, cotton, sugar beet, tomato, wheat, and muskmelon cultivars PMR 45 and Honey Dew); vertical lines represent 2 × the standard errors.

rates reported here will be affected only if experimental errors differentially affect the values obtained for the four fungi. We assumed the magnitude of any differential error to be small. In absolute terms, colonization rates reported here are similar to those reported for other fungi (11).

Another source of error in calculating colonization rates will be introduced if multiple infections are counted as single colonies. This leads to an underestimate of the actual number of infections. In most cases, colonies were well separated on roots, and a multiple-infection transformation (7) would have had a negligible effect on the data. However, in the greenhouse experiment conducted with naturally infested soil (Table 1), both *F. o. melonis* and nonpathogenic *F. oxysporum* occurred at high enough levels on some plants for more than one infection to have been counted as a single colony. Thus, for these two fungi, colonization rates in naturally infested soil in the greenhouse may have been underestimated.

Environmental influences on colonization were not tested, but several observations suggest that they may be important. For example, under greenhouse conditions, higher temperatures correlated with lower frequencies of *F. o. melonis* (and other *Fusarium* spp.) on sugar-beet roots. Significant experiment × treatment interactions suggest that environmental factors differentially influence colonization frequencies of the four fungi. This may explain why *F. equiseti* had a higher colonization rate, relative to other *Fusarium* spp., in the field than in greenhouse experiments.

The ability to colonize nonsusceptible crops undoubtedly contributes to the persistence of a soilborne pathogen. Persistence also will be influenced by the rate at which colonized tissue decomposes. Thus, pathogen colonization of the taproot, which represents the most massive and heavily lignified portion of a plant's root system, could enhance the longevity of inoculum produced on a nonsusceptible crop.

In this study, *F. o. melonis* did not differ greatly from *F. solani*, *F. equiseti*, and nonpathogenic *F. oxysporum* in ability to colonize taproots of field-grown plants. The relative abundance of these fungi in rhizosphere soils was consistent with this interpretation. Only on muskmelon cultivar PMR 45 did *F. o. melonis* appear to have a competitive advantage. The very high number of colony-forming units isolated from PMR 45 taproots and associated soil probably is a consequence of systemic infection. In most cases, this was confirmed by isolating *F. o. melonis* from stem tissue at or above the cotyledonary node.

Although relatively few fungi other than *Fusarium* spp. were isolated from taproots, *Fusarium* spp. were distinctly in the minority in rhizoshpere soil. Given their selective advantage on the isolation medium, this observation suggests that *Fusarium* spp. generally are not a prominent component of the mycoflora in the rhizosphere of taproots of crops included in this study. This is consistent with the findings of Banihashemi and deZeeuw (2) who reported low population levels of both *F. o. melonis* and nonpathogenic *F. oxysporum* in corn and soybean rhizospheres, as contrasted with high levels of other soil fungi.

The influence of crop rotation on Fusarium wilt diseases has received considerable attention, especially where resistant cultivars are not available. In general, disease control through inoculum management has been very difficult to achieve, which, in part, may be attributable to the broad adaptation of most formae speciales to the colonization of plant roots. For example, Banihashemi and deZeeuw (2) reported that *F. o. melonis* could be recovered from roots of both soybean and corn, and Smith and Snyder (22) recovered high levels of *F. o. vasinfectum* from barley grown in rotation with cotton. Katan (13) found *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. associated with resistant tomato cultivars and several weed species under field conditions, and Elmer and Lacy (4) reported that *F. oxysporum* f. sp. *apii* (Nels. & Sherb.) Snyd. & Hans. could be recovered from a number of different crop species under field conditions. Similarly, *F. o. melonis* is capable of colonizing roots on a broad taxonomic range of plants. More importantly, *F. o. melonis* is nearly as efficient as nonpathogenic strains of *F. oxysporum* as a colonizer of nonsusceptible crops. Consequently, in a field where Fusarium wilt is a problem, *F. o. melonis* seems as likely to persist as a nonpathogenic strain of *F. oxysporum*.

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