

Rhizosphere Competence of *Fusarium* Species Colonizing Corn Roots

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ABSTRACT

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We examined colonization of corn roots and rhizosphere soil by *Fusarium* species, corn root and rhizosphere colonization by *Fusarium graminearum* in competition with *F. oxysporum* or *F. proliferatum* in the growth chamber, and variability in corn root and rhizosphere colonization among isolates of *F. graminearum* and *F. oxysporum*. When corn kernels were infested with individual *Fusarium* species then planted in the field, *F. moniliforme*, *F. oxysporum*, and *F. proliferatum* were isolated from root tissue and rhizosphere soil along the entire portion of root sampled. *F. solani* was isolated from nearly the entire root length and accompanying

rhizosphere soil. *F. equiseti* and *F. graminearum*, however, were isolated from a very limited portion of the root and rhizosphere soil. Colonization patterns in a growth chamber were similar to the field study. Moreover, *F. graminearum* colonized a smaller portion of the root and rhizosphere in the presence of *F. oxysporum* or *F. proliferatum* than alone. Isolates of *F. graminearum* and *F. oxysporum* varied in degrees of root and rhizosphere colonization. We suggest that *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, and *F. solani* are rhizosphere competent on corn, whereas *F. graminearum* and *F. equiseti* are not rhizosphere competent.

Additional keywords: maize, root rot, stalk rot, *Zea mays*.

Fusarium species have been isolated and identified from corn (*Zea mays* L.) roots or rhizosphere soil in Minnesota (11,16,21). The rhizosphere competence of these species, as defined by Schmidt (20) and expanded by Ahmad and Baker (1), has been studied only briefly (7,8,15), however, and not in relation to pathogenicity. *Fusarium equiseti* (Corda) Sacc., *F. moniliforme* J. Sheld., *F. oxysporum* Schlechtend.:Fr., *F. proliferatum* (T. Matsushima) Nirenberg, and *F. solani* (Mart.) Appel & Wollenweb. have been isolated from corn roots (6). *F. proliferatum* was reported as a pathogen of corn roots (10) but did not cause root rot of corn seedlings when plants were inoculated in vitro (C. M. Ocamb and T. Kommedahl, unpublished data). *F. oxysporum* and *F. solani* have been implicated in corn stalk rot (6) but pathogenicity may depend in part on host stress (21). *F. graminearum* Schwabe is not isolated frequently from corn roots but is an important pathogen of ears and stalks, as is *F. moniliforme* (6). The isolates of *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, and *F. solani* used in our studies caused root rot when petri dish-grown corn seedlings were

inoculated (C. M. Ocamb and T. Kommedahl, unpublished data). The frequency of isolation of these species from roots and the diseases they cause on corn may be attributed to their relative rhizosphere competence.

Our objectives were to evaluate colonization of corn roots and rhizosphere soil by *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, and *F. solani* in the field and growth chamber to determine whether each species is rhizosphere competent on corn; observe corn root and rhizosphere colonization by *F. graminearum* in competition with *F. oxysporum* or *F. proliferatum* in the growth chamber; and determine variability in colonization of corn roots or rhizospheres among isolates of *F. graminearum* and *F. oxysporum* in a controlled environment.

MATERIALS AND METHODS

Inoculum production. All fungi used in this study were isolated in Minnesota. *Fusarium equiseti* (E1) and *F. proliferatum* (P1) were isolated from corn roots and *F. moniliforme* (M1) and *F. solani* (S1) from corn stalks. Five isolates of *F. graminearum* (G1-G5) and four of *F. oxysporum* (O1-O4) were included. Isolates G1, G5, and O1 were from corn roots; G4 and O3 were from corn stalks; G2, G3, and O4 were from wheat roots; and

O2 was from field soil. Isolates were purified by the single-spore or hyphal-tip method and stored at 5 C on silica gel (22). Isolates were grown on potato-dextrose agar (PDA) (5) under fluorescent lamps (three General Electric or Sylvania 40-W tubes) supplemented with black light (one Sylvania 40-W tube, BLB series) for a 12-h photoperiod at room temperature (24 C). Inoculum was increased by placing 5-mm squares of cultures grown on PDA into 50 ml of Difco (Difco Laboratories, Detroit, MI) potato-dextrose broth (PDB), which was then incubated at 24 C on a rotary shaker (60 rpm) located in a laboratory; no supplemental lighting was provided. Spore/mycelial slurries were made either by centrifugation or vacuum filtration of minced 12- to 16-day-old PDB cultures.

Seed treatments in field studies. Corn kernels of Jacques hybrid 2750 (courtesy of Jacques Seed Co., Prescott, WI) were surface-disinfested by soaking them for 5 min in 0.5% NaOCl. Then kernels were immersed for approximately 2 h in a mycelial/spore slurry of *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. proliferatum*, *F. graminearum*, or *F. equiseti*; a single isolate of each species was tested. After soaking kernels, they were lifted from slurry with sterile forceps and placed in small, paper envelopes for transport to the planting area. Ten kernels were removed from each envelope, and each kernel was individually placed in 5 ml of sterile water. Kernels were agitated for 2 min, and the numbers of propagules per kernel were determined with hemacytometer counts. Approximately 1.16×10^7 propagules per kernel were found. Kernels also were infested with spores of *Chaetomium globosum* Kunze:Fr., a potential biocontrol agent, by placing 8 g of *C. globosum* ascospores, collected from cultures grown on PDA and stored at 2 C, in a glass container. Kernels were added to the jar, gently shaken for 2 min, and poured onto a screen to remove excess ascospores. Spore concentrations per kernel averaged 3×10^3 and were determined with a hemacytometer. Controls consisted of nontreated kernels (dry control), kernels immersed in sterile distilled water for 2 h (wet control), and kernels coated with captan (75 WP). Kernels were treated with captan (75 WP) by adding 5 g of the fungicide to a glass jar; then kernels were added to the jar, gently shaken for 2 min, and poured onto a screen to remove excess captan.

Kernels were planted 5 cm deep in a sandy loam soil (pH 6.8, 3.1–4.5% organic matter) on the experimental farm at St. Paul, MN, in a field cropped with corn since 1984. The planting dates were 3 May 1988 and 17 May 1989. Treatments were assigned to plots in a randomized complete block design, and two blocks were planted on each date. Each plot contained a single row. Rows were 3 m long with 15 kernels planted per row at 20.5-cm spacings; rows were 0.91 m apart. Blocks were separated by a 1-m border. Kernels also were planted on 24 May 1989 at the Southern Experiment Station, Waseca, MN, in a field of Nicolet Webster soil (pH 6.9, 6% organic matter) cropped with soybean in 1988, corn from 1987 through 1985, and soybean in 1984. The experimental design and field layout was the same as in the St. Paul field.

Seed treatments in growth-chamber studies. Corn kernels of Jacques hybrid 2750 were surface-disinfested with 0.5% NaOCl for 10 min and were immersed for 1 h in sterile distilled water or a mycelial/spore slurry of *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, or *F. solani*. Numbers of propagules per kernel were determined with hemacytometer counts, and approximately 1.25×10^7 propagules per kernel were found. Clay pots (10.3 cm diameter) were autoclaved for 1 h at 121 C and filled with a steam-pasteurized sandy loam soil (pH 7.3, bulk density 1.04 g cm^{-3}).

Colonization of the rhizosphere and roots by *F. graminearum* when kernels infested with this fungus were planted into soil infested by *F. oxysporum* or *F. proliferatum* were examined in a growth chamber. *F. oxysporum* and *F. proliferatum* were transferred individually from PDA cultures to autoclaved cornmeal-sand mixtures (97 g of sand, 3 g of cornmeal, 40 ml of distilled water) in 250-ml flasks and incubated for 1 mo at 24 C under the light regime previously described. Cornmeal-sand cultures were dried thoroughly in a Labguard laminar flow biological

safety cabinet (NuAire, Inc., Minneapolis, MN). Dried inoculum was mixed thoroughly into the soil medium to a final concentration of 1 g of dried inoculum per 100 cm^3 of soil. One-gram samples of each infested soil were evaluated for propagule numbers of either *F. oxysporum* or *F. proliferatum* by conducting a dilution series; 1-ml aliquots were pipetted into plastic petri dishes, and 20 ml of molten Nash and Snyder's pentachloronitrobenzene-peptone agar (12) supplemented with aureomycin (11) (Nash medium) was added to each dish. Each petri dish was swirled to distribute the soil suspension evenly in the medium. Clay pots (10.3 cm diameter) were filled with infested soil, and three kernels of Jacques hybrid 2750 infested with *F. graminearum* were planted 1 cm deep in each pot.

In the growth chamber, the soil mixture was initially adjusted to -30 J/kg , and no additional water was added during the experiment. The Soil Survey Laboratory of the University of Minnesota, St. Paul, determined the gravimetric water content of the soil at -30 J/kg . Gravimetric measurements were made to determine soil moisture content and the amount of water necessary to adjust the matric potential to -30 J/kg . After addition of water, soil was allowed to equilibrate for 72 h at 24 C. A randomized complete block design was used in all growth-chamber studies; there were two blocks with one pot per treatment in each block. Polystyrene sleeves were fitted snugly (but not sealed) over the pots to minimize evapotranspiration. Pots were incubated in a Conviron growth chamber (Conviron Inc., Asheville, NC) under controlled relative humidity of 95% at 25 C light periods and 22 C dark periods (12 h/12 h). Sixteen F72T12 CW/VHO lamps and 10 60 W incandescent frosted lamps provided illumination at $688 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. One growth chamber was used for all studies. Plants were sampled 8 days after seeding.

Root and rhizosphere soil colonization measurements. In the field, five plants were randomly chosen and removed from each plot at the four- to six-leaf stage, 22–33 days after planting. Excess soil was removed by gently shaking each plant; the soil that remained was the rhizosphere soil. All rhizosphere soil was removed from roots by washing them with water. After removal of rhizosphere soil from roots, the primary root and two seminal roots (9) were removed from each plant, surface-disinfested in 0.5% NaOCl for 30 s, and placed on Nash medium.

In the 1989 field experiments, rhizosphere soil was collected from the upper 9 cm of primary root (or the entire primary root if length was less than 9 cm) from kernels infested with a *Fusarium* species or soaked in sterile distilled water. The upper 9 cm of the primary roots was cut into three 3-cm-long segments. Each primary root segment was rubbed gently with a rubber policeman in 20 ml of sterile distilled water. One milliliter of the rhizosphere soil suspension was pipetted into a plastic petri dish, and 20 ml of molten, cooled Nash medium was added. Each petri dish was swirled to distribute the soil suspension evenly in the medium.

Plants seeded in the growth chamber were excavated at the two-leaf stage, 8 days after planting. Excess soil was removed by gently shaking each of the plants from each pot. The primary root and two seminal roots were selected for assay from two plants from each pot (two pots per treatment). From one plant, excess soil was removed, and roots were transferred to solidified Nash medium. Roots from the second plant were washed under running tap water until all visible soil was removed, and roots were surface-disinfested in 0.5% NaOCl for 30 s and placed in warm, not-quite-solidified Nash medium, which eased the insertion of the roots into the medium. Rhizosphere soil was collected from the third plant in each pot. The upper 9 cm of the primary root was cut into three 3-cm-long segments. Each primary root segment was rubbed gently with a rubber policeman in 20 ml of sterile distilled water. One milliliter of the rhizosphere soil suspension was pipetted into a plastic petri dish, and 20 ml of molten, cooled Nash medium was added. Each petri dish was swirled to distribute the soil suspension evenly in the medium.

Identification of *Fusarium* species. Plates for isolation of *Fusarium* species from roots, root segments, or rhizosphere soil were incubated for at least 1 wk at 24 C without direct lighting.

Colonies of *Fusarium* species were transferred to acidified PDA and carnation leaf agar (13) media and incubated at 24 C for 2–4 wk under the light regime previously described. Some plates were stored at 5 C after 1 wk of incubation because of the large number of isolates to be identified. Each colony was examined microscopically and identified according to the system of Nelson et al (13).

Data analysis. Each experiment was done three times, except the rhizosphere soil assay for *Fusarium* species from field-grown plants, which was done twice. Data were analyzed with the SAS (19) general linear model analysis; means were compared with Tukey's W statistic ($P = 0.05$). The mean number of colony-forming units (cfu) of each *Fusarium* species per root, mean root length, mean number of cfu per centimeter of root, and mean number of cfu present in rhizosphere soil were computed within each trial for each root type (primary or seminal) or rhizosphere soil sample in each treatment and block. Data from field experiments were tested for significance due to year, site, treatment, block, root type, and interactive effects. Data from growth-chamber studies were tested for significant effects attributed to experimental run, treatment, block, root type, and interactive effects.

RESULTS

Colonization of roots and rhizospheres in the field. *Fusarium oxysporum* and *F. proliferatum* were the most prevalent species

isolated from roots and rhizosphere soil (a total of 1,295 and 818 colonies, respectively). *F. moniliforme*, *F. solani*, *F. equiseti*, and *F. graminearum* accounted for 321, 304, 51, and 15 colonies, respectively. Year had a significant effect but constituted a small portion of the model sum-of-squares, whereas site and block effects were nonsignificant; therefore, overall means (combining years and sites) were used to compare treatment effects on mean number of cfu per root, cfu per centimeter of root, mean root length, and cfu in rhizosphere soil. The mean number of cfu of each *Fusarium* species tended to be greatest in samples obtained from kernels infested with the specific species. In all six replications (two blocks per each of three dates), we observed similar patterns of colonization.

The mean number of *F. oxysporum* cfu isolated from primary or seminal roots was significantly greater when kernels were infested with this species (Fig. 1A) relative to all other plants. Recovery of *F. oxysporum* was lowest from kernels coated with captan, *F. graminearum*, or *F. solani*. The mean number of *F. proliferatum* cfu was significantly greater when kernels were infested with *F. proliferatum* or *F. oxysporum* (Fig. 1B) compared to all other kernels; no *F. proliferatum* was isolated from seedlings from either the captan- or the *F. moniliforme*-treated kernels. The mean number of *F. moniliforme* cfu per root was greatest from plants grown from *F. moniliforme*-infested kernels (Fig. 1C). Similarly, kernels infested with *F. solani* yielded roots with the greatest number of *F. solani* cfu (Fig. 1D) compared to means obtained from kernels otherwise treated. Few *F. equiseti* cfu (Fig.

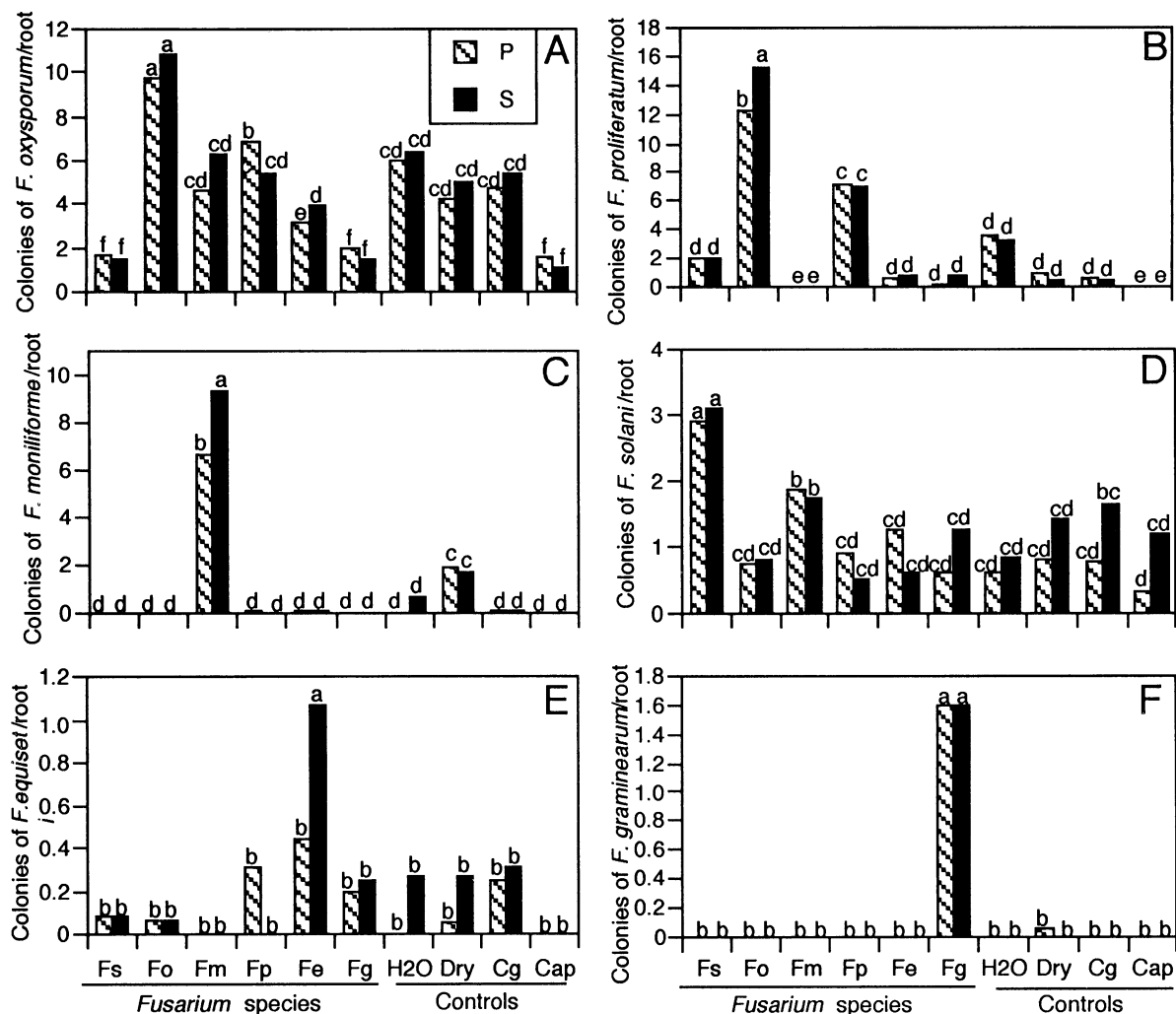


Fig. 1. Mean number of *Fusarium* colonies isolated from the primary (P) and seminal (S) roots of corn seedlings grown from kernels infested with *F. solani* (Fs), *F. oxysporum* (Fo), *F. moniliforme* (Fm), *F. proliferatum* (Fp), *F. equiseti* (Fe), *F. graminearum* (Fg), or *Chaetomium globosum* (Cg), water control (H₂O), nontreated control (Dry), or coated with captan 75 WP (Cap) and planted in the field. Means in each figure are based on 10 plants per kernel treatment averaged over three site-years (30 plants total) and were compared with Tukey's W statistic. For each graph, bars labeled with the same letter are not significantly different ($P = 0.05$).

1E) were obtained from any seedlings, but the seminal roots obtained from kernels infested with *F. equiseti* yielded significantly ($P = 0.05$) greater numbers of cfu of this fungus than any other roots sampled. Almost no cfu of *F. graminearum* (Fig. 1F) were isolated except when kernels were infested with this species.

For some species, there were significant differences in number of cfu isolated from primary roots versus number isolated from seminal roots. There were significantly greater numbers of *F. moniliforme* and *F. equiseti* cfu isolated from the seminal roots compared to the primary roots from kernels infested with these respective species, while the greatest number of cfu of *F. pro-*

liferatum was isolated from seminal roots of seedlings that grew from kernels infested with *F. oxysporum*.

Primary roots were significantly shorter ($P = 0.05$) when kernels were infested with a fungus or immersed in water prior to planting, compared to nontreated or captan-coated kernels (Fig. 2). When kernels were soaked in water or coated with spores of *C. globosum* or *F. equiseti*, shorter seminal roots resulted compared to other seedlings obtained from kernels that underwent any other treatment. The mean number of cfu of each species per root type depended on the root length available for colonization, so the mean number of cfu per species per centimeter of root length also was used for evaluating root colonization by the six *Fusarium* species. For each *Fusarium* species, the greatest mean number of cfu per centimeter of root generally was found on the primary root from kernels infested with that species (Table 1). The density of *F. oxysporum* was significantly ($P = 0.05$) greater with primary roots of seedlings from kernels infested with *F. oxysporum* or soaked in water than were *F. oxysporum* densities obtained from the other seedlings, except when kernels were infested with *F. proliferatum*. Mean numbers of *F. proliferatum* cfu per centimeter of root were greatest on primary or seminal roots of seedlings from *F. oxysporum*-infested kernels and on primary roots of seedlings from kernels treated with *F. proliferatum* compared to the means obtained from kernels otherwise treated. Mean numbers of cfu per centimeter of root of *F. moniliforme* or *F. equiseti* were significantly greater on primary and seminal roots of seedlings from kernels infested with each respective fungus compared to means obtained from all other seedlings. Mean numbers of *F. solani* cfu per centimeter of root were significantly greater on primary and seminal roots grown from kernels infested with this species, relative to all other kernels, except when kernels were infested with *F. moniliforme*. Mean numbers of *F. graminearum* cfu per centimeter of root were greatest on primary roots of seedlings from kernels infested with this fungus compared to means obtained from all other seedlings.

For each *Fusarium* species, no significant ($P = 0.05$) differences were observed among the mean number of cfu isolated from the rhizosphere soil. Colonies of *F. oxysporum* were found in the rhizosphere soil of all root segments; the colony number by root segment (0–3, 3–6, or 6–9 cm) was averaged for each treatment and ranged from 0.50 to 2.33 cfu/g of oven-dried rhizosphere soil. *F. proliferatum* was isolated from all root segments except those from kernels infested with *F. moniliforme* and the most

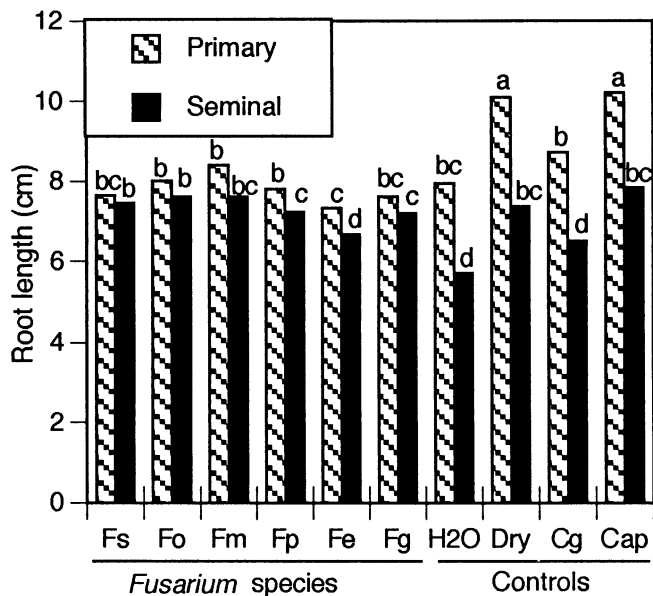


Fig. 2. Mean length of primary and seminal roots of corn seedlings grown from kernels infested with *Fusarium solani* (Fs), *F. oxysporum* (Fo), *F. moniliforme* (Fm), *F. proliferatum* (Fp), *F. equiseti* (Fe), *F. graminearum* (Fg), or *Chaetomium globosum* (Cg), water control (H₂O), nontreated dry control (Dry), or coated with captan 75 WP (Cap) and planted in the field. Means are based on 10 plants per kernel treatment averaged over three site-years (30 plants total). Means were compared with Tukey's W statistic. Bars labeled with the same letter are not significantly different ($P = 0.05$).

TABLE 1. Effect of treating corn kernels with six *Fusarium* species, *Chaetomium globosum*, captan, or water on number of *Fusarium* colonies subsequently isolated from roots (field studies)

Treatment ^x	Root type ^y	Colonies of <i>Fusarium</i> spp. per centimeter of root length ^z					
		Fo	Fs	Fm	Fp	Fe	Fg
Fs	P	0.09 def	0.15 a	0.00 c	0.10 cd	0.01 bc	0.00 c
	S	0.05 ef	0.11 ab	0.00 c	0.07 cd	0.01 bc	0.00 c
Fo	P	0.50 a	0.03 cde	0.00 c	0.62 a	0.01 bc	0.00 c
	S	0.24 bcd	0.02 de	0.00 c	0.71 a	0.00 c	0.00 c
Fm	P	0.20 bcdef	0.09 abc	0.30 a	0.00 d	0.00 c	0.00 c
	S	0.16 cdef	0.08 bcde	0.26 a	0.00 d	0.00 c	0.00 c
Fp	P	0.34 ab	0.04 cde	0.01 c	0.37 b	0.01 bc	0.00 c
	S	0.14 cdef	0.02 de	0.00 c	0.18 c	0.00 c	0.00 c
Fe	P	0.21 bcdef	0.08 bcd	0.01 c	0.03 d	0.02 ab	0.00 c
	S	0.18 bcdef	0.02 de	0.01 c	0.03 d	0.03 a	0.00 c
Fg	P	0.11 cdef	0.03 cde	0.00 c	0.01 d	0.01 bc	0.08 a
	S	0.05 ef	0.04 cde	0.00 c	0.02 d	0.01 bc	0.05 b
H ₂ O	P	0.46 a	0.02 de	0.01 c	0.36 b	0.00 c	0.00 c
	S	0.27 bc	0.03 cde	0.02 b	0.16 c	0.01 bc	0.00 c
Dry	P	0.20 bcdef	0.05 cde	0.08 b	0.05 d	0.01 bc	0.01 c
	S	0.13 cdef	0.03 cde	0.04 b	0.01 d	0.01 bc	0.00 c
Cg	P	0.24 bcd	0.04 cde	0.01 c	0.03 d	0.01 bc	0.00 c
	S	0.18 cdef	0.06 bcde	0.02 b	0.02 d	0.01 bc	0.00 c
Cap	P	0.07 ef	0.01 e	0.00 c	0.00 d	0.00 c	0.00 c
	S	0.04 f	0.05 bcde	0.01 c	0.00 d	0.00 c	0.00 c

^x Treatments included infesting kernels with *F. solani* (Fs), *F. oxysporum* (Fo), *F. moniliforme* (Fm), *F. proliferatum* (Fp), *F. equiseti* (Fe), *F. graminearum* (Fg), water control (H₂O), nontreated control (Dry), *C. globosum* (Cg), and captan 75 WP (Cap).

^y Means for the primary (P) and seminal (S) roots are based on 30 seedlings per treatment averaged over three site-years.

^z For each column, values labeled with the same letters are not significantly different ($P = 0.05$) according to Tukey's W statistic.

distal root segment sampled (6–9 cm) from kernels infested with *F. graminearum*; average colony number ranged from 0.06 to 2.57 cfu/g of oven-dried rhizosphere soil. *F. solani* cfu were isolated from most root segments, except the 3- to 6- and 6- to 9-cm segments from kernels infested with *F. graminearum* or *F. proliferatum*; the 3- to 6-cm segments from kernels infested with *F. oxysporum*; and the 0- to 3-cm segments from kernels infested with *F. moniliforme*. The average number of colonies ranged from 0.03 to 1.00 cfu/g of oven-dried rhizosphere soil. Colony-forming units of *F. moniliforme* were isolated from the rhizosphere soil of all three root segments of the primary roots from kernels infested with *F. equiseti*, *F. graminearum*, *F. solani*, and from the kernels soaked in sterile distilled water. Colony-forming units of *F. moniliforme* were isolated only from the rhizosphere soil of the 0- to 3- and 6- to 9-cm segments of the primary roots from kernels infested with *F. moniliforme* and *F. oxysporum*, respectively. The average numbers of cfu ranged from 0.05 to 1.21 cfu/g of oven-dried rhizosphere soil. Colony-forming units of *F. equiseti* were isolated only from the rhizosphere soil of the 0- to 3-, 3- to 6-, and 6- to 9-cm segments of the primary roots from kernels infested with *F. moniliforme*, *F. oxysporum*, and kernels soaked in water, and the average number of cfu was 0.10, 0.08, and 0.03 cfu/g of oven-dried rhizosphere soil, respectively. No cfu of *F. graminearum* were isolated from the rhizosphere soil.

Colonization of roots and rhizospheres in the growth chamber.

Primary roots of seedlings from kernels infested with *F. graminearum* were slightly shorter than the control roots, whereas there were no differences among mean primary and seminal root lengths from the other seedlings (Fig. 3). We expected that washing away the rhizosphere soil would reduce the subsequent numbers of cfu that grew from the roots because we expected that rhizosphere-soil colonization would contribute to the numbers of cfu that could be recovered from the roots. Perhaps a fungus colonizing the rhizosphere is susceptible to mortal injury when the rhizosphere soil, which the fungus is occupying, is removed from the plant root. Assaying nonwashed roots could give a measure of rhizosphere colonization that cannot be reflected when "disturbed" rhizosphere soil is assayed and offers an alternative by which to search the rhizosphere for an elusive fungus. Non-washed primary roots of seedlings from kernels infested with *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, or *F. solani* yielded significantly ($P=0.05$) greater numbers of *Fusarium* cfu than were obtained from washed roots (Fig. 4A). When kernels

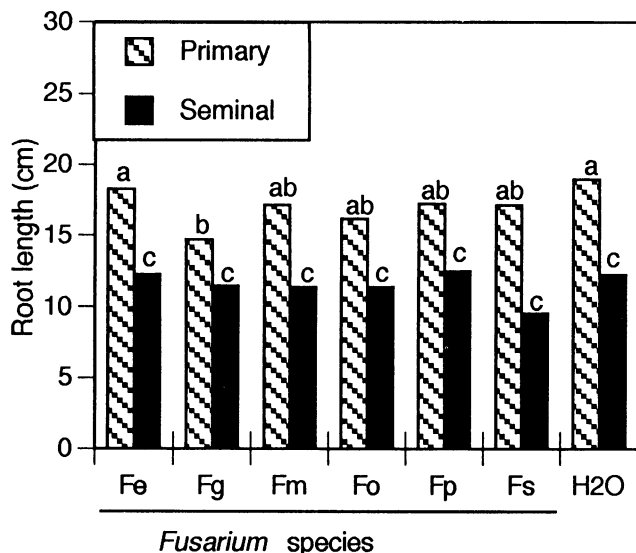


Fig. 3. Mean length of primary and seminal roots of corn seedlings grown from kernels infested with *Fusarium equiseti* (Fe), *F. graminearum* (Fg), *F. moniliforme* (Fm), *F. oxysporum* (Fo), *F. proliferatum* (Fp), *F. solani* (Fs), or water control (H₂O) and planted in a growth chamber. Means are based on two plants per kernel treatment averaged over three runs (six plants total) and were compared with Tukey's W statistic. Bars labeled with the same letter are not significantly different ($P=0.05$).

were infested with *F. graminearum*, there was no significant difference in the number of cfu obtained from nonwashed and washed roots, and these means were significantly less than the means obtained from other nonwashed primary roots. Rhizosphere soil from seedling roots from kernels infested with *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, or *F. solani* yielded *Fusarium* cfu (Fig. 4B), but *F. graminearum* was not detected in rhizosphere soil. The distal segment (6–9 cm) of the primary root yielded significantly ($P=0.05$) greater numbers of cfu than did root segments closer to the seed when kernels were infested with *F. moniliforme*, *F. proliferatum*, or *F. solani*.

When kernels were infested with *F. graminearum* and planted in soil infested with either *F. oxysporum* or *F. proliferatum*, the numbers of *F. graminearum* cfu per centimeter of primary root were significantly ($P=0.05$) fewer than the number from seedlings

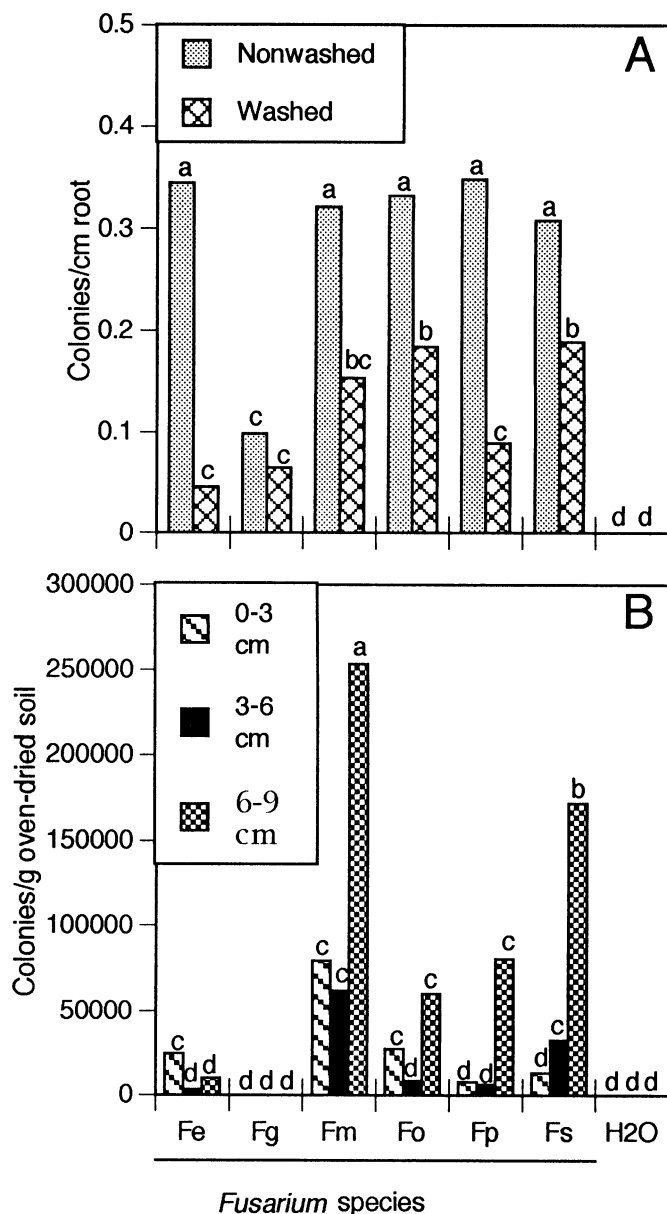


Fig. 4. *Fusarium* colonies isolated from the A, primary roots of corn seedlings or B, rhizosphere soil when kernels were infested with *F. equiseti* (Fe), *F. graminearum* (Fg), *F. moniliforme* (Fm), *F. oxysporum* (Fo), *F. proliferatum* (Fp), *F. solani* (Fs), or soaked in water (H₂O). All visible soil was removed from washed roots; rhizosphere soil was that which adhered to nonwashed roots. Means in each graph are based on two plants per kernel treatment averaged over three runs (six plants total) grown in a growth chamber and were compared with Tukey's W statistic. Bars labeled with the same letter are not significantly different ($P=0.05$).

planted in noninfested soil (Fig. 5). There also was variation among isolates of *F. graminearum* in numbers of cfu per centimeter of root (Fig. 6A), as well as among isolates of *F. oxysporum* (Fig. 6B) when kernels were infested with these *Fusarium* isolates. Isolates of *F. graminearum* (G2 and G3) and *F. oxysporum* (O4) obtained from wheat roots were able to colonize corn roots and rhizosphere soil.

DISCUSSION

Schmidt (20) defined rhizosphere competence as the consistent association of rhizobia with legume root nodules. Ahmad and Baker (1) used the concept of rhizosphere competence to describe colonization of the rhizosphere in terms of time and space; *Trichoderma* species that did not colonize the rhizosphere to a depth greater than 2 cm were not classified as rhizosphere competent, whereas the rhizosphere-competent species were found along the entire 8 cm of root-length sampled. The presence of *Fusarium* propagules in rhizosphere soil at depths greater than 2 cm is evidence that these *Fusarium* isolates are rhizosphere competent *sensu* Ahmad and Baker (1). Our studies demonstrated the first evidence of the rhizosphere-colonization abilities of these six *Fusarium* species on a single host species. In fact, rhizosphere competence of any *Fusarium* species has been only briefly reported (7,8,15). When *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, or *F. solani* was applied to a corn kernel in our field and growth-chamber studies, each species was routinely isolated from the primary and seminal roots and from rhizosphere soil of primary root segments at 3–6 and/or 6–9 cm depths. Thus, in our study, isolates of these four species were rhizosphere competent on corn when applied to kernels. Because *F. graminearum* was virtually absent from rhizosphere soil sampled in the field and growth-chamber studies, even when applied to the kernel, we do not consider this fungus to be rhizosphere competent on corn. Although *F. equiseti* was routinely isolated from rhizosphere soil in the growth chamber, we do not consider this fungus to be rhizosphere competent in our studies because the isolate used did not colonize the rhizosphere in the field. We also found that isolates from another host species (wheat) are able to colonize the corn rhizosphere. Our results confirm earlier reports that *F. solani* (21), *F. moniliforme* (16), *F. graminearum* (11,16), and

F. equiseti (11) colonize corn roots or rhizosphere soil, but *F. oxysporum* is often the most prevalent *Fusarium* species on corn roots (11,21).

Application of *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, or *F. solani* to corn kernels resulted in isolation of greater numbers of respective *Fusarium* cfu from the primary or seminal roots grown in the field, compared to kernels not treated with *Fusarium* propagules. This suggests that placement of an isolate of a rhizosphere-competent *Fusarium* species on corn kernels puts that species in a competitive position that allows that species to colonize a greater share of the root than if the kernel had not been infested. Whether the colonies obtained from the roots or rhizosphere were the same isolates applied to the kernels is conjecture; however, the results from growth-chamber studies suggest that when isolates of *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, and *F. solani* are applied to kernels,

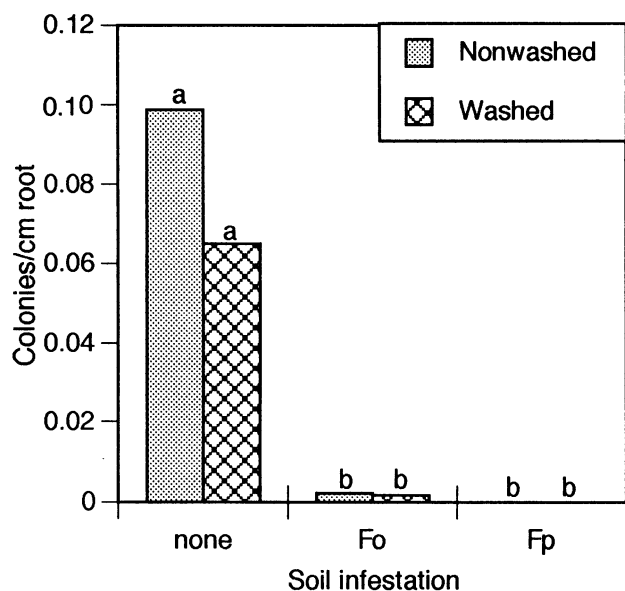


Fig. 5. *Fusarium graminearum* colonies isolated from roots from *F. graminearum*-infested kernels planted in noninfested steamed soil (none) or steamed soil infested with *F. oxysporum* (Fo) or *F. proliferatum* (Fp). All visible soil was removed from washed roots; rhizosphere soil was that which adhered to nonwashed roots. Means are based on three plants per soil treatment averaged over three runs (nine plants total) grown in a growth chamber and were compared with Tukey's W statistic. Bars labeled with the same letter are not significantly different ($P = 0.05$).

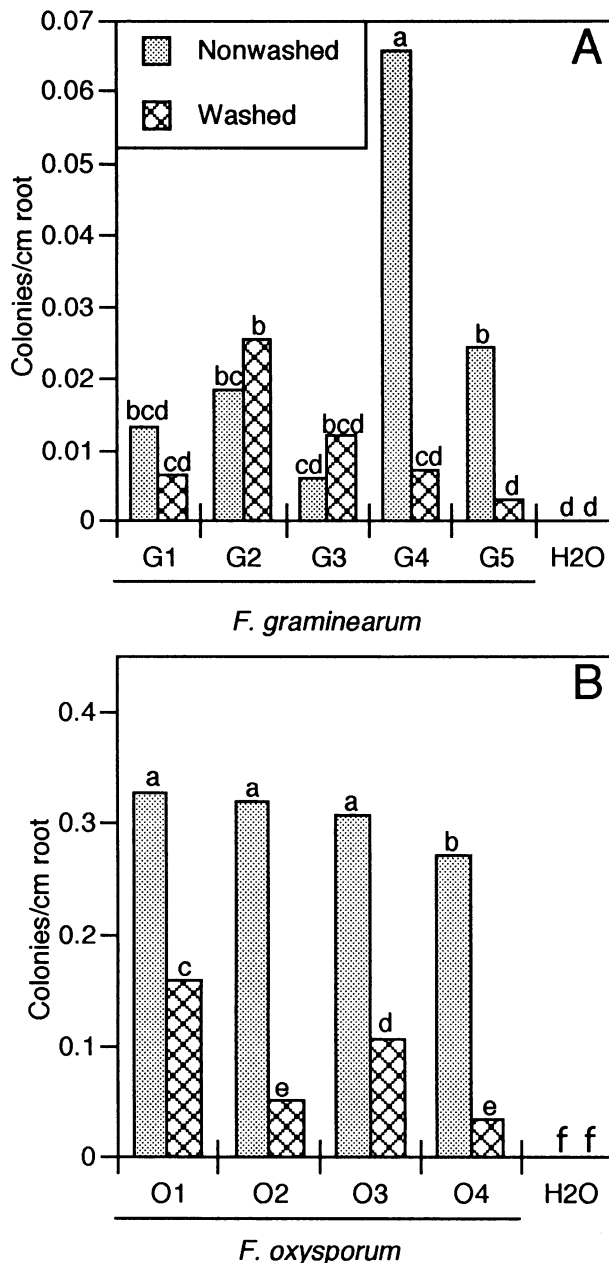


Fig. 6. *Fusarium* colonies isolated from the primary roots of corn seedlings after kernels were infested with A, *F. graminearum* (G1–G5), B, *F. oxysporum* (O1–O4), or soaked in water (H₂O). All visible soil was removed from washed roots; rhizosphere soil was that which adhered to nonwashed roots. Means in each figure are based on three plants per kernel treatment averaged over three runs (nine plants total) grown in a growth chamber and were compared with Tukey's W statistic. Bars labeled with the same letter are not significantly different ($P = 0.05$).

these isolates colonize the top 9 cm of the primary root. Molecular or immunological techniques are needed to prove this in the field. Probably not all colonies isolated from field-grown roots originated from inoculum applied to kernels. The prevalence of *F. oxysporum* along the roots and in the rhizosphere may be attributed to an ability to grow, reproduce, and survive by colonizing and parasitizing host and nonhost plants as well as functioning as a pioneer colonizer of stubble, debris, or dying plants (17). The relatively low population of *F. graminearum* may be attributed to dependence on competitive colonization of root surfaces in the absence of a suitable host (14). The reproductive ability of the fungus also may be a factor in colonization of the rhizosphere. *F. equiseti* and *F. graminearum* produce few or no microconidia, only macroconidia and chlamydospores. Perhaps rhizosphere competence of the other *Fusarium* species is due in part to their ability to produce microconidia.

The importance of knowing whether *Fusarium* species isolated from corn are rhizosphere competent or incompetent is relevant to understanding their roles as saprophytes, parasites, and pathogens, as well as evaluating their potential as biological control agents. When a *Fusarium* species is rhizosphere competent, it may grow saprophytically and reproduce in the rhizosphere. *Fusarium* species are opportunistic fungi, causing root rot when the host plant is under stress (21). Rhizosphere-competent *Fusarium* species would have an advantage, both in position and in inoculum concentration, for invasion of root tissue whenever plants are stressed. This emphasizes the importance of pathogen-free seed or protecting seed and the entire root system for control of disease. Our results show that when a rhizosphere-competent *Fusarium* isolate is present on the kernel, greater colonization of the rhizosphere occurs than if the kernel is not infested with *Fusarium*; this attribute could be important when microbes are selected for biological control. If a soilborne pathogen enters the host through roots, then an antagonist that grows along newly developed roots may offer protection from soilborne pathogens.

Our results showed that application of *F. oxysporum* to kernels enhanced root colonization by *F. proliferatum*, whereas antagonistic interactions may occur between *F. moniliforme* and *F. proliferatum* or *F. graminearum* and *F. oxysporum*. More work is needed to determine whether infestation of a kernel with these species promotes or inhibits root colonization by the other species. *F. graminearum* may have been a weak competitor with *F. oxysporum* or *F. proliferatum* in this study. Similarly, *F. equiseti* may be unable to colonize the root and rhizosphere of corn plants in the field due to the presence of competitors/antagonists. A similar limitation of colonization in the rhizosphere of pea was described for *Trichoderma harzianum* (3). Increased growth by *T. harzianum* occurred when infested seeds were planted in sterile soil compared to seeds planted in raw field soil; this limitation of *Trichoderma* colonization was attributed to an inability to compete with microflora (3).

Recovery of the various *Fusarium* species from roots obtained from kernels coated with captan suggests that species respond differently to this fungicide; *F. oxysporum* and *F. solani* were seemingly unaffected by captan. These two fungi are important pathogens of many economically important plant species, and although captan treatment of seed is common, it may be ineffective for controlling these particular pathogens. Effect of seed treatment with *C. globosum* was similar to that by captan, as previously reported (23).

We have demonstrated that variability in rhizosphere colonization exists among and within species of *Fusarium*. However, future work should examine root and rhizosphere colonization throughout the growing season because population structures may change as hosts mature. In addition, large numbers of *Fusarium*

isolates should be evaluated. Because colonization of the rhizosphere by a fungus is influenced by host and environmental factors and their interactions (2,4,18), variable rhizosphere competence of individual isolates of *Fusarium* may be demonstrated in the future. Obviously, more work must be done before rhizosphere competence can be fully utilized in protecting plants from disease.

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