Effects of Crop Residues and Colonization of Plant Tissues on Propagule Survival and Soil Populations of *Fusarium oxysporum* f. sp. *apii* Race 2

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ABSTRACT

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Laboratory-grown conidia and chlamydospores of *Fusarium oxysporum* f. sp. *apii* race 2 persisted in soil-filled nylon bags for 2 yr in fallow organic soil, but populations had declined to 10% of the initial density of 1×10^6 colony-forming units of *F. o.* f. sp. *apii* per gram of soil. Colonization of highly susceptible and moderately resistant celery plant tissues by *F. o.* f. sp. *apii* in naturally infested soils after 3 mo was about equal in feeder roots, primary roots, and crown tissue but was greater in the aboveground tissues of the highly susceptible plants. Symptomless root colonization by a pathogenic orange-colored mutant was greater in species of monocots and in carrot than in susceptible celery. In a field soil naturally infested with *F. o.* f. sp. *apii*, colonization of roots of sweet corn, cabbage, onion, lamb's-quarters, smartweed, barnyardgrass, and purslane was demonstrated. Soil populations of the orange mutant were suppressed in soil supplemented with residues of onion or mint, but celery-supplemented soils contained densities of the orange mutant isolate that were equal to the non-residue-supplemented soil for the 3-wk period. Fusarium yellows severity in celery increased in plants grown in soils supplemented with celery residues but was lower in celery grown in soils supplemented with onion residues. Root exudates of 11 crop plants stimulated chlamydospore germination equally in a chlamydospore-agar-soil overlay system. Aqueous extracts from onion residue caused greater germination than extracts from celery, mint, rye, or sudax residues.

Additional key words: disease management, soilborne disease.

Fusarium yellows of celery (*Apium graveolens* var. *dulce* (Pers.) Miller) incited by *Fusarium oxysporum* (Schlect.) emend. Snyd. & Hans. f. sp. *apii* (R. Nels. & Sherb.) race 2 (*F. o.* f. sp. *apii*) has recently become the most destructive disease of celery in Michigan (6). Soil fungicides and fumigants have been ineffective in controlling the disease (18), and acceptable highly resistant cultivars are not available (9,22). Therefore, relocation to noninfested fields or growing moderately resistant celery cultivars, such as Deacon and Tall Utah 52-70 HK, have provided the only alternatives for control.

Pathogenic formae speciales of *F. oxysporum* persist in soil for long periods of time in the absence of a host crop (2,27,28). This high capacity for survival may be due in part to the ability of the Fusarium wilt pathogens to colonize roots and stems of nonsusceptible hosts (2,13,16). Monitoring populations of pathogenic strains of *F. oxysporum* in fallow soils without any plant roots or residues has been mostly limited to greenhouse (10) and laboratory (3) studies where environmental parameters were controlled. Likewise, data on the survival of the Fusarium wilt pathogens in natural soils in the field were often clouded by the difficulty of accurately distinguishing the pathogenic forms from morphologically identical saprophytes (14).

Populations of *F. o.* f. sp. *apii* increased on infected celery roots and petioles (23) and on celery trimmings from the packing shed that are routinely incorporated into the soil of celery fields after harvest (7). Thus soil populations may continue to increase until disease levels make the currently available cultivars unprofitable, as was demonstrated with other Fusarium wilt pathogens on crops with moderate resistance (4,20). Celery is grown in Michigan exclusively on valuable organic soils, very often in a monocropping system. Infested fields that become unprofitable for celery production are not likely to be left fallow but rotated into alternate or cover crops. Because *F. o.* f. sp. *apii* has the ability to compete saprophytically for plant residues (23), information on the influence of alternate crops on soil populations of *F. o.* f. sp. *apii* could aid in planning crop rotation strategies to decrease disease severity when infested fields are rotated back into celery.

The objectives of this work were to determine the persistence of *F. o.* f. sp. *apii* in fallow soil, to study the colonization of plant tissues by *F. o.* f. sp. *apii*, to determine the effect of crop residues on soil populations of *F. o.* f. sp. *apii* and disease development, and to determine the effects of crop residues and root exudates on chlamydospore germination. All references to *F. o.* f. sp. *apii* refer to race 2 of the pathogen (26).

MATERIALS AND METHODS

Soil and plant assays for *F. oxysporum*. Soils were assayed for *F. oxysporum* by placing 5-g soil samples (dry weight basis) in 500 ml of distilled water and stirring with a magnetic stirrer bar for 30 min. Ten milliliters of the suspension was diluted with 90 ml of distilled water from which three to five replicate 5-ml aliquots were mixed into 50 ml each of molten cooled (48–50 C) Komada's selective medium (KM) (17). Five plates were poured (11 ml each) from each replicate and incubated for 10–14 days at 25 C under cool-white fluorescent lights (12-hr photoperiod). *F. oxysporum*-like colonies were single-spored onto carnation leaf agar and identified by spore morphology (21). The total number of *F. oxysporum* colony-forming units growing on the five replicate plates was recorded and expressed as number of colony-forming units (cfu) per gram of soil.

Colonization of plant roots by *F. oxysporum* was assessed by washing roots in running tap water for 2 hr, surface-sterilizing roots in 0.53% sodium hypochlorite for 1 min, and rinsing three times with distilled water. The roots were blotted dry on absorbent paper, weighed, and homogenized in 500 ml of sterile distilled water in a blender for 1 min. The suspension was stirred with a magnetic stirrer bar for 30 sec, and 10 ml was removed and added to 90 ml of distilled water. Three to five replicate aliquots (5 ml each) of the root suspension were mixed into 50 ml of cooled molten (48–50 C) KM, poured into five petri plates, and incubated as described before. Colonies of *F. oxysporum* were counted as before and expressed as colony-forming units per gram of fresh

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root tissue.

Stems were sampled by flaming 5-cm stem segments with 95% ethanol, peeling back the epidermal tissue, removing wedge-shaped pieces of vascular tissue, and placing these pieces on KM. More than 60 pieces of stem tissue were plated out per plant species per sampling.

Pathogenicity tests of randomly selected colonies. Pathogenicity tests to determine the proportion of isolates of *F. oxysporum* that were *F. o.* f. sp. *apii* from roots and soil dilution plates were carried out by transferring a single spore or hyphal tip from randomly selected colonies to the center of potato-dextrose agar (PDA) plates and incubating at 21–23 C for 2–3 wk under a 12-hr photoperiod. Colonized PDA plates were homogenized in 100 ml of sterile distilled water in a blender and mixed into 0.35 kg of commercial potting soil (1:1:1 of peat-perlite-vermiculite, pH 5.2). The infested soil was dispensed into two 10-cm-diameter plastic pots, and healthy 1-mo-old celery seedlings of Golden Detroit or Tall Utah 52.70 R Improved were transplanted into the soil. Seedlings transplanted into soil supplemented with PDA colonized by known virulent isolates of *F. o.* f. sp. *apii* or with noncolonized sterile PDA served as controls. Colonies that caused vascular discoloration in the roots or crowns of both the self-blanching (yellow) cultivar Golden Detroit and the green cultivar Tall Utah 52-70 R Improved were considered to be *F. o.* f. sp. *apii* (26).

Disease ratings of infected seedlings. Disease ratings were based on the percentage of vascular discoloration after 6 wk of growth in the greenhouse. A longitudinal slice was made with a knife through the centers of plant crowns, and disease severity was based on a scale of 1–6, where 1 = no disease, 2 = vascular discoloration in the primary roots only, 3 = vascular discoloration in 10% or less of the crown area, 4 = vascular discoloration in 11–25% of the crown area, 5 = vascular discoloration in 26–75% of the crown area, and 6 = vascular discoloration in 76–100% of the crown area or death of the plant.

Survival in fallow soil. Chlamydospores were produced in celery tissue by transferring agar plugs colonized by *F. o.* f. sp. *apii* with a sterile no. 3 cork borer to autoclaved celery (Florida 683) leaves and petioles in glass dishes followed by 3 wk of incubation at 25 C. Trimmings were air-dried, ground in a Wiley mill, and passed through a 3-mm-mesh sieve. The colonized residues, containing microconidia, macroconidia, and chlamydospores, were incorporated into organic soil believed to have no natural population of *F. o.* f. sp. *apii*. The celery residues were incorporated at a high level (5 g of colonized celery trimmings per 100 g of soil) so that naturally occurring morphologically identical nonpathogenic strains of *F. oxysporum* would be diluted to numbers that were insignificant during the assays. Noninfested soil was included as a control. Two-gram aliquots of the soil were placed in small nylon mesh (15 μm) bags and buried in the top 10–15 cm of galvanized cans (15 cm diameter × 20 cm high) filled with natural organic soil containing no *F. o.* f. sp. *apii*. The cans were placed 5–10 cm below the soil surface at two locations: in organic soil bordering a celery farm in North Muskegon, MI (site 1), and in a mineral soil on the Michigan State University botany farm campus in East Lansing (site 2). Sufficient drainage holes were provided to prevent water from collecting in the cans after heavy rains. At the beginning of the experiment and every 3 mo for 2 yr thereafter, three replicate bags were recovered from each site and assayed for densities of *F. oxysporum*. Fifty *F. oxysporum* isolates from soil artificially infested with colonized celery residues and from noninfested soil in nylon bags were recovered 15 mo after burial and tested for pathogenicity.

Production and characterization of the orange mutant (OM) isolate. During this study, a pathogenic mutant with a distinguishable colony characteristic was desired to obviate extensive pathogenicity tests required to identify *F. o.* f. sp. *apii*. Microconidia of Michigan *F. o.* f. sp. *apii* isolate FA-3 (ATCC 52626) (8) were exposed to ultraviolet light until a 95% kill was obtained (24). A surviving colony with constitutive orange pigment production was selected on KM and tested for pathogenicity on celery seedlings as described before. Fungal growth of the OM isolate was compared with the wild type by transferring one 4-mm-diameter PDA plug from the outer margins of an actively growing colony of either isolate FA-3 or the OM isolate into 125-ml flasks containing 20 ml of Czapek-Dox broth (29) (modified to 15 g of sucrose per liter). Flasks were incubated at 25 C, and the mycelial mats of four replicated flasks of each isolate were harvested after 2, 4, 6, and 8 days onto preweighed glass fiber filter papers. Fungal mats were washed twice with distilled water and dried at 80 C for 24 hr before weighing.

Colonization of plant tissues. Celery plants of Tall Utah 52-70 R Improved and Tall Utah 52-70 HK (susceptible and moderately resistant, respectively) were removed from a field naturally infested with *F. o.* f. sp. *apii* (73–103 cfu/g of soil) 3 mo after being transplanted. Three plants of each cultivar were divided into feeder roots, primary roots, crown tissue, petioles, and leaves. Petioles were further divided into sections. The first section included the first 10 cm from the base of the crown. The second section was from the first section up to and including the first node, and the third section consisted of the rest of the petiole. Each portion of tissue was assayed for *F. oxysporum* as described before.

Cabbage (*Brassica oleracea* L. 'Golden Acre'), onion (*Allium cepae* L. 'Spartan Banner'), sweet corn (*Zea mays* L.'Harmony'), lamb's-quarters (*Chenopodium album* L.), purslane (*Portulaca oleracea* L.), smartweed (*Polygonum pennsylvanicum* L.) barnyardgrass (*Echinochloa crusgalli* (L.) Beauv.), redroot pigweed (*Amaranthus retroflexus* L.), and prostrate pigweed (*A. blitoides* S. Wort.) were collected on three occasions from a field infested with *F. o.* f. sp. *apii* that had been rotated out of celery for 2 yr. The field had been planted entirely to onions the first year and to onions, sweet corn, and cabbage the second year. Depending on availability, 10–20 plants of each plant species were collected per sampling date and examined for signs of vascular discoloration. Roots were assayed as mentioned before.

Root colonization by *F. o.* f. sp. *apii* of plants cultivated to organic soils was studied comparatively in the greenhouse with the OM isolate in organic soil that had no natural populations of *F. o.* f. sp. *apii*. Wheat straw colonized by the OM isolate was incorporated into natural organic field soil (3 g of wheat straw per kilogram of soil) by rotating the soil in a concrete mixer for 20 min. Soil was stored in plastic bags at 30% moisture content for 6 wk at 22–25 C to allow time for the OM isolate populations to stabilize to about 6×10^3 cfu of the OM isolate per gram of soil.

The following plants were grown in the soil infested with the OM isolate: celery cultivars Tall Utah 52-70 R Improved (highly susceptible) and Tall Utah 52-70 HK (moderately resistant), onion (Spartan Banner), carrot (*Daucus carota* L. 'Gold Pak'), cabbage (Golden Acre), parsley (*Petroselinum crispum* (Mill.) Nym. 'Moss Curl'), lettuce (*Lactuca sativa* L. 'Minetto'), peppermint (*Mentha piperita* L., 'Black Mitcham"), sweet corn (Harmony), sudax (Sorghum × sudangrass), purslane, barnyardgrass, lamb's-quarters, and crabgrass (*Digitaria sanguinalis* (L.) Scop.). All plants were seeded in three replicate pots of soil infested with the OM isolate except for celery and parsley, which were placed in the soil as 1-mo-old transplants, and mint, which was transplanted as rooted 1-mo-old cuttings. Three pots for each species were prepared, each containing five seedlings per pot. All plants were incubated for 6 wk in a greenhouse at 22–25 C. Three 100-ml applications of Peter's (20-20-20) soluble fertilizer (W. R. Grace & Co.) were made at 2-wk intervals beginning 1 wk after germination or transplanting. After 6 wk, roots of each pot were excised, collected in a 1-mm-mesh sieve under a stream of water, and roots from replicate pots were combined into a composite sample for each species. Roots were washed to remove all adhering soil particles, and assayed for colonies of the OM isolate as described previously. Onion roots were so scarce that they were not homogenized but surface-disinfested and placed on KM plates. After incubation, the OM isolate colonies were counted.

Effects of crop residues on soil densities of *F. o.* f. sp. *apii* and on disease development in celery. Celery trimmings (Florida 683), small onion culls (Spartan Banner), peppermint foliage (Black Mitcham), potato vines (*Solanum tuberosum* L. 'Russet Burbank'), rye (*Secale cereale* L.), and sudax (Haygrazer) residues

collected at the Michigan State University Muck Farm in Bath were air-dried, ground, passed through a 5-mm-mesh sieve, and stored in plastic bags at 4 C. Five-hundred-gram aliquots of the soil infested with the OM isolate (30% moisture content) were supplemented with celery, onion, and mint residues at the rate of 1 g of residue per liter of soil. The residue-supplemented soils were moistened with distilled water to bring the moisture content up to 50%, and soils were incubated at 25 C in the dark in airtight plastic bags loosely tied to allow for gas exchange. Nonsupplemented soils served as controls. Five-gram soil samples (dry weight basis) were assayed for densities of OM isolate at day 0 and day 1 and at weeks 1, 2, and 3. The experiment was repeated once.

Soil naturally infested with *F. o.* f. sp. *apii* (73–103 cfu/g of soil) was dried, passed through a 2-mm-mesh sieve, and supplemented with residues of celery, onion, mint, potato, rye, or sudax (1 g of residue per liter of soil). These soils were allowed to incubate in loosely tied plastic bags under the greenhouse bench for 1 mo in the greenhouse at 22–25 C. Three 1-mo-old celery seedlings (Tall Utah 52-70 R Improved) were then transplanted into 10-cm-diameter plastic pots filled with the residue-supplemented soils. Celery seedlings were also transplanted into steamed field soil and a mixture of steamed to naturally infested field soil (1:1, v/v) supplemented with the residues. There were 10 pots per treatment and three plants per pot. Celery seedlings grown in soil not supplemented with residues served as controls. Disease ratings and dry weights were recorded at 6 wk and analyzed for differences. The experiment was repeated three times.

Effects of root exduates and aqueous crop extracts on chlamydospore germination. A modification of the soil overlay system used by Hart (11) was employed to study chlamydospore behavior in response to root exudates of various plants. Conidia of isolate FA-3 were produced on plates of carnation leaf agar (21) or potato-carrot agar (29) after 15–17 days of incubation at 25 C under a 12-hr photoperiod. Conidia were washed from the surfaces of plates with 10 ml of sterile distilled water, filtered through four layers of cheesecloth to remove mycelial fragments, and washed three times by centrifugation at 3,000 *g* for 5 min. The final resuspended pellet consisted of microconidia and macroconidia at an approximate ratio of 10:1. Macroconidia were enumerated with a hemacytometer, and the conidial suspension was mixed with cooled molten (48–50 C) water agar in an amount sufficient to yield 2.5×10^5 macroconidia per milliliter, and the agar was immediately poured into 6-cm-diameter petri plates (3 ml/plate). Chlamydospores were induced by placing 3.5 g of a mineral soil (Sphinks loamy sand) over the surface of the agar. The soil had been moistened with water 2 hr before use to yield a final moisture content of 20%. Plates were incubated for 3 days in the dark, then air-dried with the plate covers off for 2–3 days. Soil was removed from the agar surface with a paintbrush under a gentle stream of water. Chlamydospores were easily observed microscopically in the agar at 200×. They developed terminally at the ends of short macroconidial germ tubes or less frequently were intercalary within macroconidia. Chlamydospores were distinguished from hyphal swellings by the presence of thick cell walls and dense cytoplasm.

Seeds of highly susceptible and moderately resistant celery cultivars, along with seeds of carrot, parsley, cabbage, and lettuce, were germinated in a commercial potting mix, and seedlings were used after 3 wk. Corn, rye, and sudax seeds were germinated on sterile filter paper and removed after 5 days. Onion was similarly germinated and removed after 7 days. Mint cuttings were rooted in steamed sand under intermittent mist. Roots of healthy seedlings were gently washed to remove soil particles, and seedlings were placed on top of the chlamydospore-agar-soil overlay system so that the foliar portions of the seedling extended through a hole in the side of the plastic petri dish. The seedlings were anchored to the surface of the agar by placing a small quantity of soil on the stem, but the roots were left exposed. After 2 days of incubation at 25 C under a 12-hr photoperiod, the regions behind the root apices (reported to be the infection court for *F. o.* f. sp. *apii* [12]) were positioned horizontally near the bottom of a microscope field at 200×, and total germinated chlamydospores out of 25 were counted in the region above the root. The same root was moved to the top of the field, and germinated chlamydospores were counted in the field below the root. Chlamydospores were scored as germinated if the length of the germ tube was at least equal to the diameter of the spore. At least three root tips of four seedlings were examined for each plant species. Untreated chlamydospore-agar-soil overlay system plates served as controls. The experiment was repeated once with similar results.

Aqueous extracts from residues of celery, onion, mint, sudax, and rye were prepared by suspending 1 g of the dried plant residues in 100 ml of distilled water and stirring the suspension with a magnetic stirrer bar for 2–3 hr. The suspension was filtered through four layers of cheesecloth and centrifuged for 10 min at 10,000 *g*. The supernatant from each plant residue was placed in four agar wells (200 µl/well) produced in the chlamydospore-agar-soil overlay system with a no. 1 cork borer (4 mm). After a 48-hr incubation in the dark at 25 C, plates were stained with phenolic rose bengal, and the areas surrounding the agar well were examined microscopically at 200×. The number of germinated chlamydospores out of 100 counted along the perimeter of each well was recorded. The mean of four wells represented one replicate, and four replicates were examined per treatment. Wells filled with distilled water served as controls. The experiment was repeated once.

RESULTS

Survival of *F. o.* f. sp. *apii* in fallow soil. Laboratory-grown propagules of *F. o.* f. sp. *apii* persisted in the soil-filled nylon bags for the 2-yr period in site 1 (Fig. 1) and site 2. Fluctuations in the number of recovered colonies were observed during the first year. A dramatic increase in *F. oxysporum* colonies was noted in the last 3 mo of the first year. During the second year, the populations fluctuated less and had declined in a linear fashion to about 10% of the original level by the end of the year. Pathogenicity tests were conducted on isolates of *F. oxysporum* recovered from the artificially infested soil sampled 15 mo after burial. Only *F. o.* f. sp. *apii* propagules were recovered. All isolates of *F. oxysporum* recovered from the noninfested soils were nonpathogenic on celery transplants.

Characterization of the OM isolate. The OM isolate generated lower disease severity ratings on celery seedlings than the wild type isolate FA-3 but was able to incite vascular discloration and marginal stunting of celery (Table 1). The OM isolate was readily reisolated on KM and consistently yielded orange-colored colonies. When the OM isolate was compared with the wild-type isolate FA-3 for growth in Czapek-Dox broth, an initial lag in

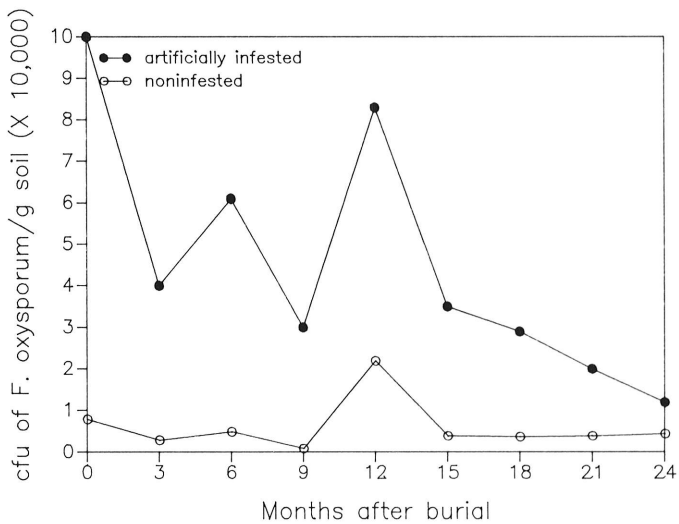

Fig. 1. Colony-forming.units (cfu) of laboratory-grown propagules of *Fusarium oxysporum* f. sp. *apii* in organic soil over time as determined by soil dilutions into Komada's selective medium.

TABLE 1. Comparison of the orange mutant (OM) isolate with wild-type isolate FA-3 of *Fusarium oxysporum* f. sp. *apii* race 2 for disease severity on celery and growth in Czapek-Dox broth

Isolate	Disease severity rating[y]	Dry weight (g)	Dry weight (mg) increase in Czapek-Dox[z] broth over time (days)			
			2	4	6	8
OM isolate	3.4 b	1.7 ab	0.3 a	3.0 a	14.0 a	21.3 a
FA-3	5.1 c	1.4 b	0.8 b	7.3 b	16.6 a	23.0 a
Untreated	1.0 a	2.1 a	...	...	...	...

[y] Disease rating based on the scale: 1 = no disease, 2 = vascular discoloration in the primary roots only, 3 = vascular discoloration in 10% or less of the crown area, 4 = vascular discoloration in 11–25% of the crown area, 5 = vascular discoloration in 26–75% of the crown area, and 6 = vascular discoloration in 76–100% of the crown area or death of the plant; values represent the mean of five pots, with three plants per pot. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[z] Represents the mean of four fungal dry weights grown at 25 C in 20 ml of Czapek-Dox broth modified to 15 g sucrose. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

TABLE 2. Distribution of *Fusarium oxysporum* in celery plants naturally infected with *F. oxysporum* f. sp. *apii* race 2

Tissue	Colony-forming units of *F. oxysporum* per gram of fresh tissue	
	Tall Utah 52-70 R[w] (HS)	Tall Utah 52-70 HK[w] (MR)
Feeder roots	614 b[x]	419 b
Primary roots	1,610 c	1,460 c
Crown	282 b	264 b
Petioles from crown to 8 cm up	>2500[y]	0 a
Petioles from 8 cm up to first node	444 b	0 a
Petioles from first node and higher	4 a	3 a
Leaves	...[z]	0 a

[w] HS = highly susceptible and MR = moderately resistant to *F. o.* f. sp. *apii*.

[x] Values represent the mean of three replicate samples of tissue. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[y] Colonies were too numerous to count; this is an estimate.

[z] Not assayed.

fungal growth was observed for the OM isolate, but differences were not detected between the two isolates after 6 days of growth. The OM isolate was adopted for short-term laboratory and greenhouse soil assays to circumvent pathogenicity tests otherwise required to identify *F. o.* f. sp. *apii*. All assays conducted on crops or weeds grown under field conditions were done in soils naturally infested with *F. o.* f. sp. *apii*.

Colonization of plant tissues. The moderately resistant cultivar (Tall Utah 52-70 HK) was colonized by *F. oxysporum* in the feeder roots, primary roots, and crown tissues in amounts approximately equal to those in the susceptible cultivar Tall Utah 52-70 R when grown in the field in naturally infested soil (Table 2). Recovered colonies of *F. oxysporum* in the belowground plant parts were significantly greater for both cultivars in the primary roots than in feeder roots or crown tissue. All of the petiole sections of the highly susceptible cultivar yielded colonies of *F. o.* f. sp. *apii*, whereas only a few colonies of *F. o.* f. sp. *apii* were detected in the upper petioles of the moderately resistant cultivar.

No vascular discoloration was observed in any of the plants that were sampled in the field infested with *F. o.* f. sp. *apii* that had been rotated out of celery 2 yr previously. However, isolates of *F. o.* f. sp. *apii* were recovered from asymptomatic roots and stems of crops and weeds (Table 3). *F. o.* f. sp. *apii* was detected in the stems of only onion, cabbage, and smartweed, whereas no *F. oxysporum* was detected in the stems of the other plant species. Barnyardgrass roots were consistently more heavily colonized by *F. oxysporum* than those of other plants (Table 3). Findings were consistent among the three sampling periods.

When plants were grown for 6 wk in soil infested with the OM isolate, colonies of the OM isolate were recovered from roots of all plants tested except parsley and crabgrass (Table 3). No visible disease symptoms occurred on any plant except in celery, where a slight vascular discoloration in the crown was observed. The number of colony-forming units of OM isolate recovered from roots of a highly susceptible celery cultivar was threefold greater than that recovered from a moderately resistant cultivar. However, there were 10-fold more colony-forming units recovered from carrot roots than from susceptible celery roots. Roots of corn and barnyardgrass yielded extremely high numbers of colonies of the OM isolate. Cabbage, mint, and sudax roots yielded colony counts of the OM isolate approximately equal to those from susceptible celery, whereas greater numbers were recovered from the roots of the weeds lamb's-quarters and purslane than from susceptible celery. The roots of parsley and crabgrass did not appear to be colonized by the OM isolate, and lettuce was only slightly colonized. Only four colonies of the OM isolate were counted from the 21 onion roots that were placed on the KM without homogenization.

Effects of crop residues on soil populations of *F. o.* f. sp. *apii*. Residues of celery, onion, and mint promoted an increase in the colonies of the OM isolate in soil 1 day after incorporation (Fig. 2). Onion residues stimulated the highest increase of up to 2×10^4 cfu of the OM isolate per gram of soil. One week after the residue incorporation, populations of the OM isolate declined to about 1/10 of the day 1 level in onion-supplemented soil and to one-fifth of the day 1 density in mint-supplemented soil. OM isolate soil densities declined about 40% from the initial densities in soil treated with celery residues and in untreated soil. Densities of the OM isolate were suppressed in the onion or mint residue-supplemented soils after 2 and 3 wk compared with the nonsupplemented soil control, but the celery-supplemented soils had populations equal to the nonsupplemented control. After 3 wk of incubation, populations of the OM isolate had declined in all treatments to similar levels.

Effects of crop residues on Fusarium yellows of celery. Dry weights of celery plants grown in mixtures of steamed and naturally infested soil (1:1, v/v) supplemented with celery residues were significantly lower than those in untreated soil (Table 4). Sudax and rye residue supplements did not significantly decrease the dry weights of celery in this trial, but repeated experiments suggested that these residues retarded celery growth. Celery plants grown in soils supplemented with celery, sudax, and rye residues had higher disease ratings than plants grown in nonsupplemented soil. Significant increases in dry weights of celery grown in onion-supplemented soil (infested-steamed soil, 1:1, v/v) and in mint-supplemented soil (100% naturally infested) were recorded, indicating that these residues had suppressed the disease, but the trend was not seen in the infested-steamed soil (1:1, v/v) mixture supplemented with mint. Onion residues seemed to suppress vascular discoloration of celery plants when grown in soil supplemented with these residues, but differences were not statistically significant (Table 4). These findings were consistent in two repetitions of the experiments. Onion residues consistently inhibited disease development more than other residues, whereas celery-supplemented soil consistently enhanced the disease.

Effects of root exudates and aqueous residue extracts of celery and nonsusceptible plants on chlamydospore germination. All

plant roots tested in the chlamydospore-agar-soil overlay system produced exudates that stimulated germination of chlamydospores (Table 5). No significant differences were observed in the percentage of germinated chlamydospores between root exudates of the highly susceptible celery cultivar and the moderately resistant one.

Chlamydospore germination occurred around all root areas but was observed to be greater 1.3 mm behind the root apices. In almost every instance, germ tubes were oriented toward the root, indicating a chemotropic stimulation. When corn and sudax seedlings were tested, chlamydospore germination was extremely high in areas of the plate far removed from the roots of corn and sudax with germ tubes oriented in a random manner. Wide variability in spore germination occurred around roots of the same plant and between roots of different plants of the same species despite care to standardize treatments.

All soluble residue extracts stimulated germination of chlamydospores in the chlamydospore-agar-soil overlay system (Table 5). Extracts from celery promoted the least spore germination, whereas onion extracts caused an average 62%

TABLE 3. Symptomless colonization of weeds and crop plants by *Fusarium oxysporum* f. sp. *apii* race 2 under field conditions in naturally infested soil and in the greenhouse in soil artificially infested with the orange mutant (OM) isolate of *F. o.* f. sp. *apii* race 2

		Naturally infested soil[s]		Artificially infested soil[s]
Plant	Tissue	Total *F. oxysporum*/g root tissue[t]	No. isolates tested/no. pathogenic[u]	Total OM isolate cfu/g fresh root[v]
Celery (HS)[w]	Roots	–[x]	...	19.1 b
Celery (MR)[w]	Roots	–[x]	...	6.6 a
Carrot	Roots	...	...	198.0 d
Parsley	Roots	...	...	0.0 a
Lettuce	Roots	...	...	1.5 a
Mint	Roots	...	...	16.4 ab
Sudax[y]	Roots	...	...	15.2 ab
Sweet corn	Roots	29.2 ab	14/7	458.7 e
Cabbage	Roots	4.5 a	3/1	13.3 ab
	Stem	...	3/1	...
Onion	Roots	15.1 ab	3/2	4.0 a[z]
	Stem	...	1/1	...
Lamb's-quarters	Roots	93.4 c	14/10	78.7 c
	Taproot	...	2/2	...
Purslane	Roots	56.6 bc	9/3	67.7 c
Smartweed	Roots	27.0 ab	18/14	...
	Stem	...	5/4	...
Barnyardgrass	Roots	485.5 d	17/1	4,093.6 f
	Stem	...	1/0	...
Crabgrass	Roots	...	...	0.0 a

[s] Naturally infested soil had about 73–103 cfu of *F. o.* f. sp. *apii* per gram of soil; wheat straw colonized by the OM isolate had a final inoculum density of 6×10^3 propagules of the OM isolate per gram of soil. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[t] Root colonization was determined by diluting homogenized root suspensions into Komada's medium, incubating the plates, and counting colonies of *F. oxysporum*. Stems and taproots were assayed by placing tissue segments on solidified Komada's medium, incubating the plates, and counting colonies of *F. oxysporum*.

[u] Isolates were tested for pathogenicity on celery cultivars Tall Utah 52-70 R Improved and Golden Detroit.

[v] Values represent the mean of three sample aliquots from a composite root sample of 15 plants grown in organic soil artificially infested with the OM isolate. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[w] HS = highly susceptible to *F. o.* f. sp. *apii* (Tall Utah 52-70 R Improved) and MR = moderately resistant to *F. o.* f. sp. *apii* (Tall Utah 52-70 HK).

[x] Data presented in Table 1.

[y] Sorghum × sudangrass.

[z] Onion roots were not comminuted but placed on KM after surface sterilization and represented four colonies of the OM isolate from 21 roots.

TABLE 4. Effects of crop residues on celery dry weights and disease ratings of celery grown in different levels of soil naturally infested with *Fusarium oxysporum* f. sp. *apii* race 2

	Percent naturally infested soil[w]					
	0		50		100	
Residue[v]	DR[x]	DW[y]	DR	DW	DR	DW
No residues	1.0 a[z]	4.4 a	3.4 a	2.0 b	4.0 a	1.4 b
Celery	1.0 a	3.9 a	5.3 b	0.7 a	5.0 b	0.9 a
Onion	1.0 a	3.4 a	3.3 a	2.4 c	3.5 a	1.6 b
Mint	1.0 a	3.7 a	4.5 a	1.6 b	3.2 a	2.4 c
Potato	1.0 a	3.0 a	3.6 a	2.3 bc	3.5 a	1.6 b
Rye	1.0 a	3.3 a	4.7 a	1.4 ab	4.4 a	1.7 b
Sudax	1.0 a	4.0 a	4.8 ab	1.1 ab	4.8 ab	1.1 a

[v] Residues were incorporated at 1 g of dried residue per liter of soil.

[w] Naturally infested soil had 73–103 cfu of *F. o.* f. sp. *apii* per gram of soil, determined by conventional soil dilutions and pathogenicity test, and was mixed with steamed soil to achieve ratios of 0–100%.

[x] Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration in the primary roots only, 3 = vascular discoloration in 10% or less of the crown area, 4 = vascular discoloration in 11–25% of the crown area, 5 = vascular discoloration in 26–75% of the crown area, and 6 = vascular discoloration in 76–100% of the crown area or death of the plant.

[y] Dry weight (g) of the foliar plant portion.

[z] Values represent the mean of 12 pots consisting a three plants per pot. Values followed by different letters are significantly different from their respective control according to Duncan's multiple range test at $P = 0.05$.

germination, which was significantly greater than with other extracts. Mint and sudax extracts stimulated chlamydospore germination more than celery root exudates.

DISCUSSION

Laboratory-produced propagules of *F. o.* f. sp. *apii* persisted for 2 yr in fallow soils but declined 90% from the initial density. The wide fluctuation in propagule recovery during the first year was probably the result of sampling error. However, the initial propagule density decrease during the first 3 mo was also observed in soil studies with *F. oxysporum* f. sp. *melonis* (Leach & Currence) (31). This phenomenon could partially be explained by the loss of nonpersistent microconidia (19). Using high artificial inoculum densities allowed us to dilute out the natural levels of nonpathogenic strains of *F. oxysporum* to insignificant amounts and forego the pathogencity tests required to identify *F. o.* f. sp. *apii*. However, because the mortality rate of naturally occurring propagules at natural levels may be different from those reported here, extrapolation of a mortality curve from our data may not reflect the actual field mortality rate.

The colonization of nonsusceptible plant roots probably plays an important role in the extended survival of *F. o.* f. sp. *apii* as suggested for other wilt *Fusarium* species (2,13,16,27). It is doubtful that short-term fallowing of infested fields would provide any effective disease control unless inoculum densities were already very low, because even low inoculum densities can cause crop failure at harvest (5,30).

The differences observed in the recovered colonies of the OM isolate from the roots of the various plant species are probably a reflection of different intensities of colonization. The greater root colonization by the OM isolate of the highly susceptible celery cultivar than in the moderately resistant cultivar suggests that the resistance of Tall Utah 52-70 HK lies in its ability to slow the colonization of the roots by the pathogen and restrict it to the crown. In naturally infested soils, the upper petioles of susceptible celery were invaded, which demonstrated the ability of *F. o.* f. sp. *apii* to colonize systemically and its potential for rapid spread to noninfested soils when these colonized celery trimmings are returned to the soil after harvest.

Using the OM isolate, as described by Schneider (25), eliminated the need for pathogenicity tests that were required for identification of *F. o.* f. sp. *apii* and allowed for rapid enumeration of *F. o.* f. sp. *apii* propagules. However, because the OM isolate was lower in virulence on celery and possessed a slightly lower growth rate than the wild-type isolate FA-3, results must be interpreted with caution. Greenhouse and field studies using natural populations should support these findings before they are finally accepted.

Celery roots were expected to be relatively highly colonized by the OM isolate, but it appeared that extent of root colonization was actually higher in barnyardgrass, corn, and carrot, because more colonies were recovered. This was also observed for plants grown in soil naturally infested with *F. o.* f. sp. *apii*. The pathogen may have a preference for colonizing roots of certain nonsusceptible plants. Katan (16) demonstrated that *F. oxysporum* f. sp. *lycopersici* (Sacc.) colonized the roots of gramineous monocots more heavily than those of dicots, which (with the exception of crabgrass) concurred with our findings. Likewise, Smith and Snyder (27) discovered that *F. oxysporum* f. sp. *vasinfectum* (Atk.) would increase faster in soils planted to barley, a gramineous monocot, than in soils with continuous cotton.

The ability of the OM isolate to colonize different plant residues was variable. The initial stimulation of the OM isolate in onion residue-supplemented soil (Fig. 2) and chlamydospore germination in onion residue extracts in the chlamydospore-agar-soil overlay (Table 5) system could have been a direct response to the high soluble carbohydrate level in onion tissue (15). However, *Allium* spp. have also been reported to produce compounds toxic to several plant-pathogenic fungi (1,32). The sharp decline in the plate counts of the OM isolate after 1 wk of incubation may have been due to similar compounds.

The celery and mint residues did not generate as much increase in the OM isolate populations (Fig. 2) or chlamydospore germination (Table 5) as did onion residues. Additionally, mint residues appeared to adversely affect persistence of the OM isolate. Because recovered OM isolate colonies in all residue-supplemented soils were not significantly different from the nonsupplemented soils after 3 wk, the residue effect appeared to be short term. We are not certain whether this was artifactual resulting from the use of the OM isolate or whether other factors were not controlled. Nevertheless, the initial effect of the residues on the OM isolate was observed again upon repetition of the experiment. The actual mechanisms governing this phenomenon remain unclear.

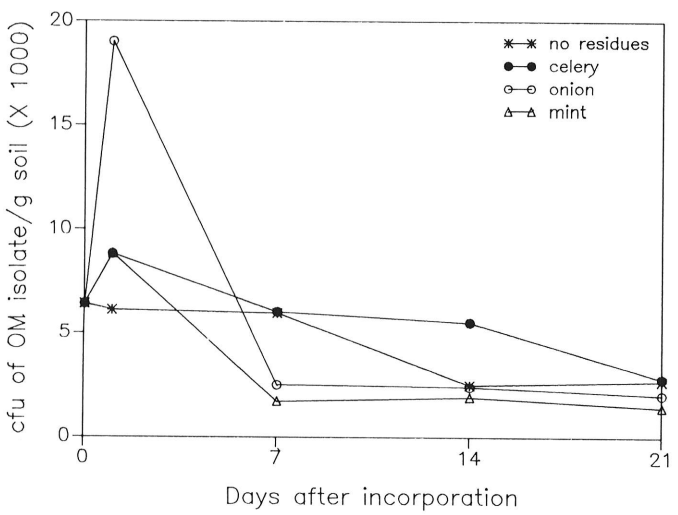

Fig. 2. Effects of soil-incorporated crop residues on colony-forming units (cfu) of an orange-colored mutant (OM isolate) of *Fusarium oxysporum* f. sp. *apii* over time as determined by soil dilutions into Komada's selective medium.

TABLE 5. Effects of root exudates and aqueous residue extracts on chlamydospore germination of *Fusarium oxysporum* f. sp. *apii* race 2 in a chlamydospore-agar-soil overlay system

Plant	Percent chlamydospore germination in the chlamydospore-agar-soil overlay system[w]	
	Root exudates[x]	Aqueous extracts[y]
Control	1.6 a	3.7 a
Celery (HS)[z]	48.2 b	4.5 a
Celery (MR)[z]	61.0 b	···
Carrot	25.8 b	···
Parsley	34.5 b	···
Onion	43.5 b	61.8 c
Cabbage	19.1 b	···
Lettuce	28.0 b	···
Mint	27.0 b	25.4 b
Rye	64.8 b	9.0 ab
Corn	97.0 c	···
Sudax	80.4 bc	29.6 b

[w] Chlamydospore-agar-overlay system was produced by inducing chlamydospores from conidia in water agar treated with a soil overlay.

[x] Values represent the mean of four samples consisting of three root apices counted per sample. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[y] Values represent the mean of three replicates, each consisting of four samples of 50 chlamydospores produced in the chlamydospore-agar-soil overlay system. Values followed by different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

[z] HS = highly susceptible to *F. oxysporum* f. sp. *apii* (Tall Utah 52-70 R Improved) and MR = moderately resistant to *F. oxysporum* f. sp. *apii* (Tall Utah 52-70 HK).

The chlamydospore-agar-soil overlay system may have generated propagules with a different nutritional status from what would have occurred under natural conditions, so a direct comparison of these findings with field situations may not warranted. However, the nonspecificity of the various plant root exudates on chlamydospore germination would be expected in view of the great number of symptomless hosts discovered in the field. The high percentage of chlamydospore germination in response to corn and sudax exudates could have resulted from carbohydrate leakage from the attached seeds, because chlamydospore germination occurred at distances far from the plant roots and germ tube orientation was random.

Disease suppression or enhancement resulting from residue supplements may be solely a response of the residue's effect(s) on populations of *F. o.* f. sp. *apii*. Our findings suggested that continuous celery cultivation will cause an increase in *F. o.* f. sp. *apii* densities from decaying infected celery roots and colonized celery trimmings returned to the soil after harvest, resulting in enhanced disease development. Alternate crop plants such as corn or carrots, along with the gramineous cover crops and weeds, may encourage soil densities of *F. o.* f. sp. *apii* to increase and therefore negate the benefit of crop rotation. Rotation with onions, however, may prevent populations of *F. o.* f. sp. *apii* from increasing as fast as with the other alternate or cover crops so that disease severity may be lessen when infested fields are planted back into celery with moderately resistant cultivars. Additional crop rotation experiments are needed to validate this hypothesis.

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