Effects of Inoculum Densities of *Fusarium oxysporum* f. sp. *apii* in Organic Soil on Disease Expression in Celery

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ABSTRACT

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Populations of *Fusarium oxysporum* f. sp. *apii* in naturally infested organic soil were enumerated by conventional soil dilution methods and subsequent pathogenicity tests. Celery plants grown in naturally infested soil and either diluted or not diluted with autoclaved soil showed reductions in mean dry weights and increases in disease severity ratings that were proportional to estimated inoculum density in each dilution of soil. Infection incidence, correcting for multiple infections [$\log_e 1/(1-X)$], increased with inoculum density in a curvilinear fashion. An inoculum density of 42 propagules per gram of field soil was predicted to incite sufficient infections to cause vascular discoloration in every plant within 6 wk. A mean of 36.5 propagules per gram of soil was necessary to incite significant growth reductions within this period. Vertical samples of organic soil naturally infested with *F. o.* f. sp. *apii* contained the highest inoculum densities in the 15- to 30-cm profile, which corresponded to the zone of greatest root density. Propagule densities decreased significantly at soil depths below 45 cm.

Fusarium yellows of celery (*Apium graveolens* L. var. *dulce* (Pers.) Miller) is incited by *Fusarium oxysporum* (Schlect.) emend. Snyd. & Hans. f. sp. *apii* (R. Nels. & Sherb.) (*F. o.* f. sp. *apii*). Fusarium yellows was reported in Michigan celery fields in 1982 after an absence of about 25 yr and by 1984 had been identified on 11 commercial celery farms that had no history of the disease (4,5). By the second year after the disease first appeared, growers experienced 50–100% disease incidence in infested fields. These recent outbreaks of Fusarium yellows of celery in Michigan were caused by race 2 of the pathogen (4,11), which seriously threatens celery production because of ineffective control measures and a lack of highly resistant cultivars (6).

Welch (13) postulated that inoculum levels of one propagule of *F. o.* f. sp. *apii* per gram of mineral soil would incite severe disease in celery by harvest and that an inoculum density of above 2.8 propagules per gram of soil would result in 100% disease incidence after 14 wk. It was not known if these predictions would apply to the disease situation in the organic soils in Michigan where celery is grown. Organic soils lack defined soil horizons, which are characteristic of most mineral soils, and differ in other properties (3). Because little information is available on vertical distribution of propagule densities of *F. o.* f. sp. *apii* in organic soils, this aspect was examined in Michigan's organic soils.

This research sought to determine the disease-causing capacity of naturally infested field soils on celery in the greenhouse and the inoculum densities in vertical profiles of a Michigan organic field soil. All references to *F. o.* f. sp. *apii* will be to race 2 of the pathogen (11).

MATERIALS AND METHODS

Enumeration and disease-causing capacity of *F. o.* f. sp. *apii* in naturally infested soil. In May and June 1984, naturally infested soil was removed with a shovel to a depth of 15 cm at 50 arbitrary locations in a celery field where disease had been severe for several years. The soil was bulked and sieved through a 2-mm-mesh screen, mixed in a concrete mixer for 20–30 min, and stored in plastic bags at room temperature at about 32% moisture for 3–5 days until assayed.

Five 5-g soil subsamples (oven-dry weight equivalent) were removed at random from the sampled field soil and assayed for *F. o.* f. sp. *apii* as described below. Sixty colonies of *F. oxysporum* (16% of the total) identified from the soil dilution plates were assayed for pathogenicity. The percentage of these isolates that was pathogenic was used to estimate the densities of *F. o.* f. sp. *apii* in naturally infested soils and was expressed as viable propagules per gram of soil.

Field soil was autoclaved for 3 hr at 121 C and stored for 1 wk before use. Autoclaved field soil was used to dilute naturally infested field soil into the following proportions of naturally infested to disinfested soil (v/v): 1, 0.75, 0.50, 0.25, 0.10, and 0. Each soil batch was mixed in a concrete mixer for 20 min, then dispensed into 12 15-cm-diameter clay pots. Three healthy 1-mo-old celery seedlings of a susceptible celery cultivar (Tall Utah 52-70 R Improved) were transplanted into each pot. The pots were arranged in the greenhouse in a randomized block design. Pots received three 100-ml applications of Peter's soluble fertilizer (20-20-20, 15 g/L) (W. R. Grace Co.) at 2-wk intervals beginning 1 wk after transplanting. Six weeks later, each plant was rated for vascular discoloration in the crown according to the scale: 1 = no disease, 2 = vascular discoloration in the primary roots only, 3 = vascular discoloration in less than 10% of the crown, 4 = vascular discoloration in 11–25% of the crown, 5 = vascular discoloration in 26–75% of the crown, and 6 = vascular discoloration in 76–100% of the crown or death of the plant. Foliar portions were removed and oven-dried to constant weights at 80 C for 24 hr, weighed, and recorded. The experiment was repeated using the second sample of infested soil collected 1 mo after the first sample.

Assay method for *F. oxysporum*. Five-gram samples (dry-weight basis) of soil were placed in 500 ml of distilled water and stirred with a magnetic stirrer bar for 30 min. Then 10 ml of the suspension was diluted with 90 ml of distilled water from which three replicate 5-ml subsamples were each mixed into 50 ml of molten,

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cooled (48–50 C) Komada's selective medium (KM) (9). 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 examined microscopically, and identification was later confirmed by spore morphology after subculture on carnation leaf agar (10). Data are presented as colony-forming units (cfu) of *F. oxysporum* per gram of soil.

Pathogenicity tests. Single spores or hyphal tips from randomly selected colonies were transferred to the center of potato-dextrose agar (PDA) plates and incubated 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, mixed into 0.35 kg of commercial potting soil (1:1:1, v/v/v, of peat-perlite-vermiculite, pH 5.2), and dispensed into two 10-cm-diameter plastic pots. Healthy 1-mo-old celery seedlings of cultivars Golden Detroit (race 1- and race 2-susceptible) or Tall Utah 52-70 R Improved (race 2-susceptible) 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 causing vascular discoloration in the roots or crowns of both cultivars were considered to be *F. o.* f. sp. *apii* race 2 (11). Colonies causing vascular discoloration in only Golden Detroit (there were none) would have been considered to be race 1.

Vertical distribution of *F. o.* f. sp. *apii* in naturally infested field soil. On 17 May and 15 September 1984, soil samples were collected with a soil-sampling probe from a celery field in North Muskegon, MI, where 100% of the plants were diseased the previous year. Six 7.6-cm-diameter × 15-cm cylindrical samples were removed consecutively down to a total depth of 90 cm. Five sites were sampled and soil cores from the same depth were combined into composite samples. Composite samples were air-dried, ground in a mortar and pestle, passed through a 40-mesh (1.4-mm) sieve, and stored in plastic bags at 21–23 C until used. All samples were assayed within 2 wk of collection. Densities of *F. o.* f. sp. *apii* in each composite sample were estimated by the soil dilution method. Because of the number of *F. oxysporum* isolates in this soil and the decreasing numbers with increasing depth, the following percentages of total isolates from each 15-cm soil section were tested for pathogenicity: 0–15 cm, 10%; 15–30 cm, 10%; 30–45 cm, 10%; 45–60 cm, 20%; 60–75 cm, 33%; and 75–90 cm, 100%. All of the *F. oxysporum* isolates were tested for pathogenicity in the second experiment conducted in September because propagule recovery was much lower than in the previous assay.

RESULTS

Enumeration and disease-causing capacity of *F. o.* f. sp. *apii* in naturally infested field soil. Several morphological types of *F. oxysporum* developed on KM after 7 days of incubation of soil dilution plates. Aerial mycelium associated with *F. oxysporum* colonies varied from sparse and low-growing to raised and fluffy. All colonies of *F. oxysporum* had a red pigmentation surrounded by a creamy white border. Colonies of known isolates of *F. o.* f. sp. *apii* were morphologically indistinguishable from other *F. oxysporum* colonies.

There was an average of 363 colonies of *F. oxysporum* per gram of soil in a heavily infested field soil. Twelve of 60 (20%) randomly selected *F. oxysporum* isolates incited mild to severe vascular discoloration on both celery cultivars in greenhouse tests; mean density of *F. o.* f. sp. *apii* was estimated to be 73 propagules per gram of soil (20% of 363). The naturally infested soils mixed with autoclaved soil in proportions described above were estimated to contain mean inoculum densities of 0, 7.3, 18.3, 36.5, 58.8, and 73.0 propagules per gram of soil after dilution with autoclaved soil. When the experiment was repeated, 100 isolates of *F. oxysporum* were assayed for pathogenicity and 42% were identified as *F. o.* f. sp. *apii*. Inoculum density in this sample of naturally infested soil was estimated to be 103 propagules of *F. o.* f. sp. *apii* per gram of soil, with densities in field soil diluted with autoclaved soil calculated as above.

The mean dry weights of celery plants grown at different inoculum densities decreased as inoculum increased (r^2 = 0.57). However, a significant decrease from the noninfested control (Student-Newman-Keuls test, $P = 0.05$) was not detected until the inoculum had reached 36.5 propagules per gram of soil (Fig. 1). Mean disease severity ratings also increased linearly as the estimated inoculum density increased (r^2 = 0.97). Vascular discoloration was detected in celery plants growing in soil containing the lowest inoculum density of 7.3 propagules per gram.

Gregory's multiple transformation [$\log_e 1/(1 - X)$] (12) can predict the number of effective root infections that result in vascular discoloration from the fraction of diseased plants (X) at each inoculum level. The transformed data regressed against the inoculum levels indicated a curvilinear relationship (r^2 = 0.99), which predicted that an average 0.18 root infections per plant would result from 10 propagules per gram of soil within a 6-wk period (Fig. 2). An inoculum density of 42 propagules per gram of soil was necessary to incite enough effective root infections to result in vascular discoloration in each crown. It would appear that vascular discoloration in the crown would significantly reduce celery plant weight within 6 wk since a significant reduction in growth was detected when a mean of 36.5 propagules per gram of soil was present (Fig. 1).

Vertical distribution of *F. o.* f. sp. *apii* in naturally infested field soil. *F. oxysporum* densities in cylindrical soil sections sampled from an infested field decreased as soil depth increased (Fig. 3). The greatest density of *F. o.* f. sp. *apii* was found at soil depths of 15–30 and 30–45 cm. The pathogen was detected in all soil samples down to 90 cm.

When the experiment was repeated in September 1984 (data not shown), *F. oxysporum* densities were 25% of the amounts observed in May of that year, but the relative numbers at different depths were similar. *F. o.* f. sp. *apii* was also lower in numbers in all soil layers except at 0-15 cm, where no significant

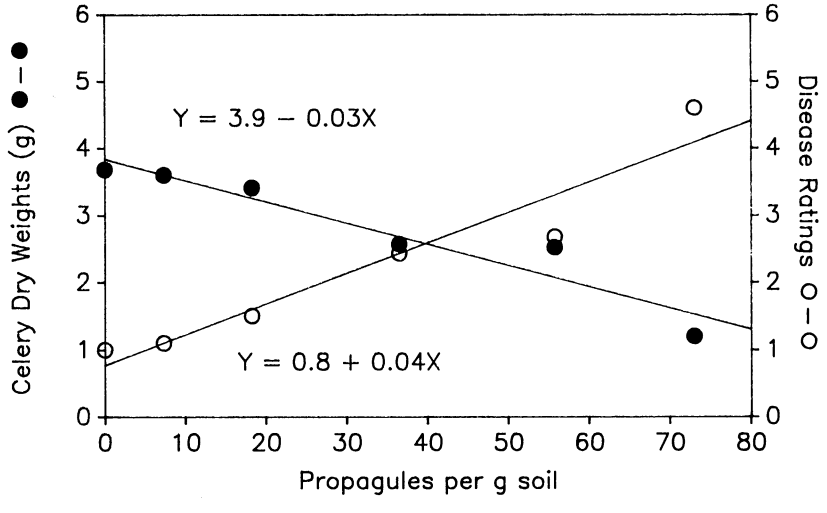

Fig. 1. Relationship of increasing inoculum densities of *Fusarium oxysporum* f. sp. *apii* to mean celery dry weights and to mean disease ratings in celery plants after 6 wk of growth in the greenhouse.

difference was detected from the first sampling (Student's t test, $P = 0.05$). No isolates obtained from the soil layers at depths of 30–45, 45–60, and 75–90 cm were pathogenic on either celery cultivar.

DISCUSSION

The hypothesis that relatively low levels of inoculum can cause major crop failure was supported by the estimation that there was a mean of 73 propagules of *F. o.* f. sp. *apii* per gram of organic soil from a field where severe disease loss was observed in recent years (13). We predicted that 0.18 root infections per plant would result from an inoculum load of 10 propagules per gram of soil after 6 wk. However, lower inoculum densities could incite the same disease level if plants were allowed to grow longer than 6 wk. Welch's (13) results indicated that the slopes of the inoculum density/infection incidence [$\log_e 1/(1 - X)$] curves of propagules of *F. o.* f. sp. *apii* per gram of soil significantly increased as the incubation period increased. Therefore, infections occurring after 6 wk would probably increase the disease severity, and the actual number of propagules required to incite severe disease by harvest would be lower. More important, bulk densities of the California mineral soils (1.25–1.45 cm^3/g) where celery is grown and the Michigan organic soil (0.2–0.3 cm^3/g) used in these assays are very different (3). Since the volume of 1 g of organic soil surrounding a suitable infection court on a celery root tip would occupy five to seven times as much space as 1 g of mineral soil, it would appear that a higher inoculum density in organic soil would be required to incite the same level of disease as in a mineral soil. Propagules in organic soil at the same inoculum density would be at a greater average distance from the infection court than in the mineral soil and therefore would be less likely to germinate in response to root exudates from the apices of celery roots (7). A volumetric measurement of inoculum density (propagules per cubic centimeter of soil) would correct for this.

The curvilinear relationship between infection incidence and inoculum densities suggests that propagules at higher inoculum densities were more efficient in inciting root infections than those at lower levels. Welch (13) observed a similar increase in infection incidence at higher inoculum densities of *F. o.* f. sp. *apii*. This increase in infection may relate to greater excretion of root exudates from diseased roots that stimulate additional germination of spores near the infection court. Another possible explanation could be that as root tips become infected and cease growth, new lateral root growth may be induced and immediately become infected because of the high inoculum density. These interpretations must be viewed with caution, however. It is possible that inoculum densities in the proportions of naturally infested soil diluted with autoclaved soil may have changed over time and resulted in an inaccurate assessment of the relationship. We also recognize that Gregory's transformation tends to overcorrect mutiple infection data at high inoculum densities and that this could have affected the relationship (12).

One must exercise caution when comparing greenhouse experiments with field responses. It has been noted that disease symptoms of chlorosis and wilt were rare in greenhouse trials (6) but commonly appeared in infected celery in the field late in the season. A typical celery growing season in Michigan lasts 8–10 wk and begins with 7- to 8-wk-old transplants that contain more roots and infection sites than the 3- to 4-wk-old seedlings used in this study. Welch (13) proposed that since celery plants possessed fibrous root systems that permeate large volumes of soil (8,14), the probability of additional infections occurring throughout the growing season is high. Thus, the actual propagules per gram of soil needed to incite a certain level of disease would decrease with longer growing periods since root infections not yet detectable as vascular discoloration would have more time to develop. Even if these infections did not cause noticeable stunting, however, any discoloration in the crown at harvest would decrease marketability.

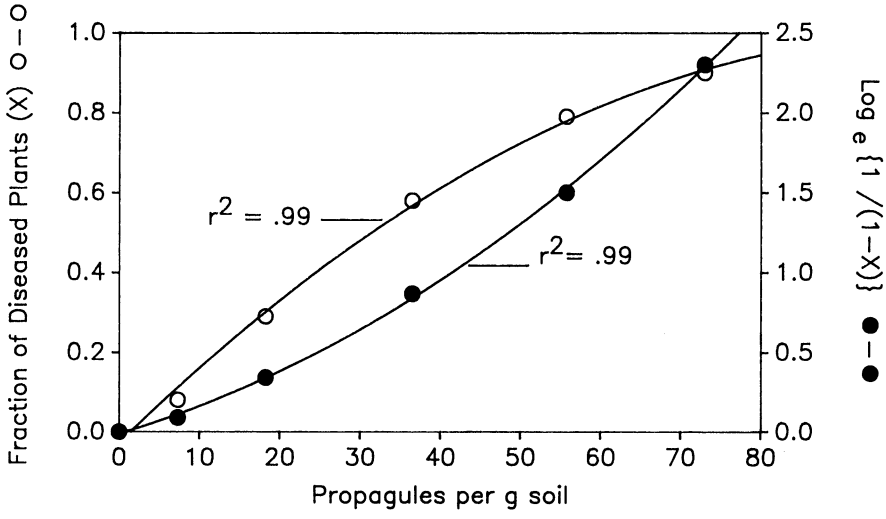

Fig. 2. Relationship of increasing inoculum densities of *Fusarium oxysporum* f. sp. *apii* to the fraction of diseased plants (X) detected and to infection incidence [$\log_e 1/(1 - X)$] of celery plants after 6 wk of growth in the greenhouse.

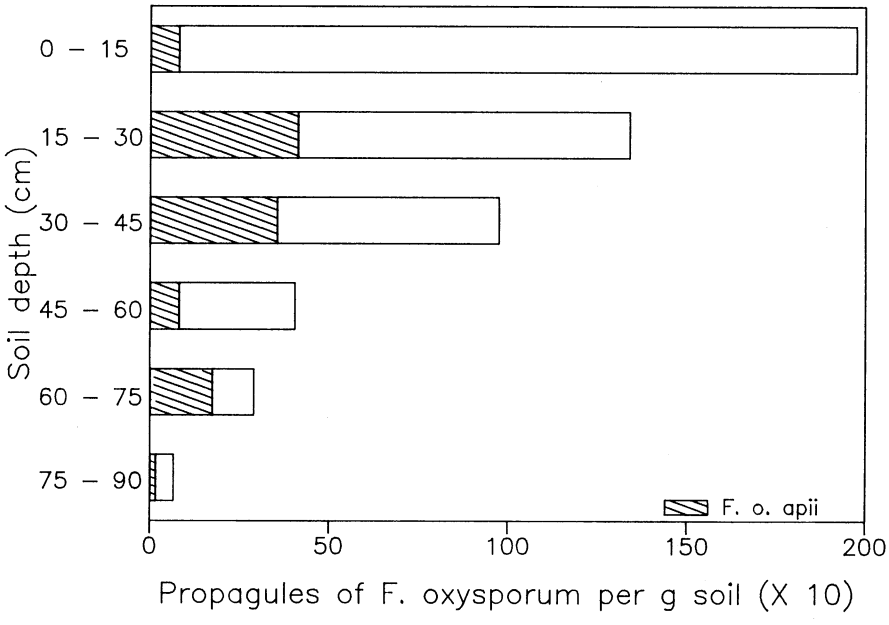

Fig. 3. Vertical distribution of *Fusarium oxysporum* and *F. oxysporum* f. sp. *apii* in naturally infested organic soil as determined by soil dilution plating and subsequent pathogenicity tests.

The highest densities of *F. o.* f. sp. *apii* estimated were observed at soil depths of 15–30 cm. This area corresponded approximately to the root zone where infected roots and crowns along with colonized celery trimmings from previous year's crop were reincorporated and left for decay. Although contamination from upper soil strata may have contributed to the detection of the pathogen at soil depths >30 cm when the soil samples were removed, it is likely that infected celery roots reach such depths in the soil. Since organic soil horizons are much less well defined than in mineral soils (3), physical soil barriers of lower horizons in organic soils are always less prominent or missing, and infected roots probably extend into lower depths. Propagules of *F. o.* f. sp. *albedensis* were detected in mineral soil 50–100 cm deep in an oil date orchard (2). Likewise, more than 1,000 propagules of *F. o.* f. sp. *cepae* per gram of soil were detected at a depth of 30–45 cm in an organic soil cropped to onion (1). However, data on celery root depth in organic soils is lacking at present to support the conclusion that infections could occur at such depths.

The sudden appearance of the disease in several of Michigan's celery-growing areas within a short time suggests that the pathogen was recently introduced into these fields. Inasmuch as low inoculum densities can result in extensive crop loss, it would seem that a relatively small amount of introduced infested soil could quickly lead to noticeable symptoms of Fusarium yellows if highly susceptible cultivars were grown. Since many commercial celery operations in Michigan share transplants and harvesting equipment, infested soil and infected transplants could have aided in disseminating the pathogen into these fields. However, the detection of Fusarium yellows in isolated disease foci in most fields, usually at the field edge, suggested that infested soil might have been introduced on farm equipment and initially contaminated the soil at the field edge. One would expect a more uniform distribution of disease symptoms within a field if infected transplants were the primary inoculum source.

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