Effects of Nonpathogenic Strains of *Fusarium oxysporum* on Celery Root Infection by *F. oxysporum* f. sp. *apii* and a Novel Use of the Lineweaver-Burk Double Reciprocal Plot Technique

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ABSTRACT

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Soils suppressive to Fusarium yellows of celery are described. Results from a greenhouse experiment in which sterilized or unsterilized suppressive and conducive soils (S and C soils, respectively) were mixed in various proportions and planted to celery, indicated that the suppressive agent was biological and did not multiply during the period of observation. This is in contrast to other S soils in which substantial disease control has been achieved following extensive dilutions and in which bacteria with short generation times have been implicated. Strains of *Fusarium oxysporum* were isolated from celery roots growing in the S soil that, when incorporated into an experimental soil along with the pathogen, effectively reduced the rate of root infection (colonies per 100 cm of root) by the pathogen. Nonpathogenic strains of *F. oxysporum* from the C soil were generally ineffective in reducing root infection by the pathogen. Determinations of suppressive ability of nonpathogenic strains of *F. oxysporum* isolated from symptomless celery roots indicated that there are large differences in this trait among populations from different areas. Reduction in root infection by the pathogen appears to be a function of the ratio of inoculum densities of effective suppressive isolates to those of the pathogen. The relationships between inoculum density of the pathogen versus rate of root infection as affected by several test isolates were analyzed by the Lineweaver-Burk double reciprocal plot technique. Results of these analyses suggested that isolates that either enhanced or suppressed root infection by the pathogen did so by affecting the susceptibility of a finite number of infection sites rather than by affecting the number of such sites. The most effective isolates reduced disease severity when added to an experimental greenhouse soil and a raw soil collected from a field with severely diseased plants.

Additional key words: biological control.

Fusarium yellows of celery (*Apium graveolens* L. var. *dulce* (Miller) Pers.), which is caused by *Fusarium oxysporum* f. sp. *apii* (R. Nelson & Sherb.) Snyder & Hans. (*Foa*), has become a serious problem in California (9). Land in areas with a suitable climate for celery culture is limited (19) and, as a result, cultural control afforded by long-term crop rotation cannot be practiced. In addition, the disease may go unnoticed for several years until inoculum density (ID) increases throughout a field to a level that causes vascular discoloration in the crown, or harvested portion of the plant, and finally, the uniform occurrence of severe stunting (29). These conditions provide opportunities to detect large areas or localized sites within fields that may be suppressive to disease

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development. Soils that are disease suppressive are those in which disease does not develop even though the pathogen and susceptible host may be present (3,21).

This report describes sites where soil is suppressive to Fusarium yellows of celery and provides evidence that implicates nonpathogenic root-infecting strains of *F. oxysporum* in the phenomenon. A preliminary report has been published (22).

MATERIALS AND METHODS

Demonstration of disease suppression. A celery field in Ventura County, CA, was uniformly devastated by Fusarium yellows except for three small islands of about 50 plants each that remained symptomless for the duration of the season (Fig. 1). Soils from the putative disease-suppressive and -conducive areas (S and C soils, respectively) were sampled from the shoulders of the beds to a depth of 20 cm with a sampling tube. The samples were air-dried, diluted in a 10-fold series in 0.1% water agar, plated on nonautoclaved Komada's medium (13), which is selective for *F. oxysporum*, and incubated at room temperature under continuous fluorescent light for 1 wk. Colony pigmentation is greatly subdued when the medium is autoclaved (*unpublished*). Stock cultures of the pathogen that were stored in dry soil were similarly plated to provide references for colony morphology (29). A total of 220 colonies from the dilution plates (10% of the reference colony types) were single-spored and tested for pathogenicity (29). Samples of S and C soil were collected from the suppressive and conducive sites and analyzed. The results, respectively, are as follows: nitrate-N, 4.8 and 7.4 μg/g; exchangeable K, 2.8 and 3.1 meq/100 g; exchangeable Ca, 8.8 and 8.6 meq/100 g; exchangeable Mg, 2.2 and 2.6 meq/100 g; bicarbonate extractable phosphate-P, 78 and 72 μg/g; pH, 6.8 and 6.8; and ID of *Foa*, 252 (±80) and 268 (±101) propagules per gram.

Additional soil cores were removed to a depth of 30 cm and the samples were soaked in 1% sodium hexametaphosphate (SHMP) for 3 hr. The roots were then sieved out of the soil and agitated for 1 hr in a fresh solution of sterile SHMP. Subsamples were either rinsed in three changes of sterile water or immersed in 0.21% sodium hypochlorite (4% household bleach) for various time intervals followed by three sterile water rinses. They were then plated on Komada's medium. After incubation for 7 days at room temperature under fluorescent light, rates of root infection (colonies per 100 cm of root) by *F. oxysporum* were determined as described previously (24). Ten percent of each of the three major colony types of *Fusarium* spp. growing from the roots were selected at random, subcultured, single-spored, identified according to Toussoun and Nelson (28), tested for pathogenicity as described below, and saved for further study.

Soil samples from each of the three suppressive areas were bulked as were samples from throughout the remainder of the field. The soils were dried at room temperature and sieved through a screen with openings of 0.6 cm. The S and C soils were then mixed in a soil blender to give final proportions of S soil of 0.0, 0.01, 0.10, 0.25, 0.50, 0.75, 0.90, 0.99, and 1.0. In addition, a subsample of S soil was autoclaved for 1 hr, and the same dilutions were repeated using sterilized S soil and raw C soil. The soils were placed in 15-cm-diameter pots and maintained at field capacity for 10 days to allow microbial populations to stabilize before being planted with 6-wk-old celery transplants (one plant per pot), cultivar Tall Utah 52-70R. The pots were fertilized twice weekly with half-strength Hoagland's solution (11). At 8 wk after transplanting, the plants were washed from the soil and rated for disease severity by the following scale: 0 = symptomless, 1 = intermittent vascular discoloration in roots, 2 = extensive vascular discoloration in roots, 3 = mild vascular discoloration in crown, 4 = severe vascular discoloration in crown, 5 = mild disintegration of crown tissue, 6 = extensive disintegration, and 7 = wilted or dead. There were 10 replications per treatment and the experiment was repeated once.

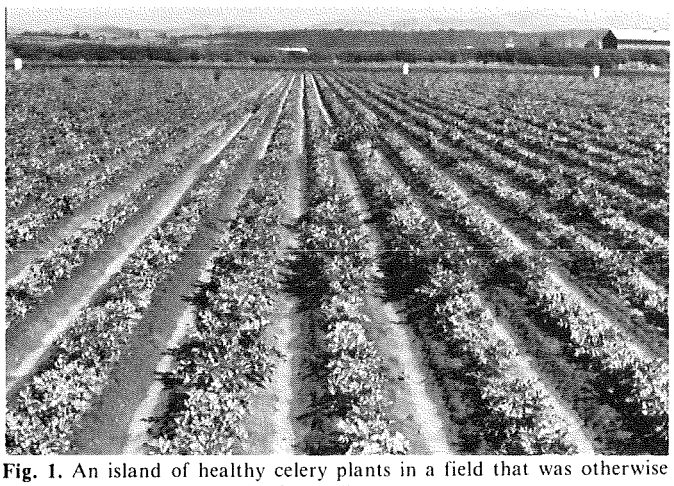

Fig. 1. An island of healthy celery plants in a field that was otherwise uniformly devastated by Fusarium yellows.

Standardization of the root infection assay. Isolate number 40-1 of *Foa* (American Type Culture Collection, No. 48420) was subjected to mutagenic ultraviolet light and selections were made for orange mutants as described by Puhalla (17). These mutants are easily recognized on Komada's medium, thus facilitating quantification of root infection. Five such mutants were obtained, single-spored, and tested for pathogenicity by the infested barley straw technique described below.

Cultures were grown in potato-dextrose broth (PDB) on a rotary shaker for 48 hr at room temperature. Dried barley straw, ground to pass through a 1.0-mm sieve, was moistened with 0.025 M L-asparagine, autoclaved for 30 min, inoculated with a conidial suspension (10^6/ml) obtained from PDB cultures, and incubated for 2 wk at room temperature. The barley straw cultures, hereafter referred to as inoculum, were dried at room temperature and refrigerated until used. Microscopic examination showed that hyphae had grown throughout the bits of barley straw, and micro- and macroconidia, but no chlamydospores, were observed in the mounting solution. However, when the barley straw was plated on potato-dextrose agar (PDA) and Komada's medium, colonies originated only from the bits of straw, which suggests that the conidia had been killed during drying. Inoculum was incorporated into an air-dry greenhouse soil mix (soil:peat:sand, 2:1:1, v/v; pH 6.8) in a soil blender at the rate of 0.5 g of inoculum per kilogram of soil. Soil infested with each test isolate was placed in pots, celery seedlings were transplanted therein, grown for 8 wk, and rated for symptoms as described above. Dry weights also were recorded. Isolate 40-1-ora 3 (*ora-3*) was chosen for further study because it was similar to the wild type in symptomatology, effect on plant growth, radial growth on PDA at 18 and 25 C, and production of microconidia in PDB (Table 1).

TABLE 1. Comparisons of five orange mutants of *Fusarium oxysporum* f. sp. *apii* and the wild type (40-1) for virulence, effect on celery growth, mycelial growth on potato-dextrose agar, and production of microconidia in potato-dextrose broth (PDB)

Isolates	DSI[x]	Plant dry weight (g)	Colony diameter (mm) 18 C	Colony diameter (mm) 25 C	Microconidia per ml ($\times 10^{-6}$)[y]
40-1	5.8 a[z]	1.6 a	36 a	59 a	5.6 a
ora-1	4.6 b	2.7 b	37 a	57 a	5.0 ab
ora-2	4.0 bc	2.6 b	37 a	57 a	4.7 b
ora-3	5.5 a	1.9 a	36 a	60 a	5.8 a
ora-4	5.5 a	2.0 a	35 a	61 a	1.2 c
ora-5	3.5 c	3.8 c	34 a	59 a	5.7 a
Uninoculated control	1.0	4.1	...	...	...

[x] Disease severity index: 1 = symptomless; 7 = wilted or dead.
[y] PDB cultures were inoculated with 1 ml of a conidial suspension containing 10^3 conidia per milliliter and grown for 36 hr.
[z] Values followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

An experiment was conducted to determine the appropriate amount of *ora-3* inoculum to incorporate into soil for further experiments. The greenhouse soil mix was amended with 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 g of inoculum per kilogram of soil and placed into pots 15 cm in diameter. Celery seedlings were transplanted into the soil (one plant per pot) and maintained as described above. After 4 wk, the roots were sieved from the soil, washed in SHMP, surface-sterilized for 4 min, rinsed in three changes of sterile water, and assayed for rate of infection as described above. No symptoms were apparent at this time and the plants were not pot-bound. There were eight replications for each level of inoculum and the experiment was repeated twice with similar results.

Comparative root infection assays. A total of 110 randomly selected isolates of *F. oxysporum*, some of which were pathogenic, were obtained from surface-sterilized roots from the suppressive and conducive sites. They were single-spored and tested for effect on *ora-3* in infecting celery roots. An additional 118 isolates of *F. oxysporum* from symptomless celery roots from four other locations throughout California were similarly tested. Sampling sites chosen included an area in which the pathogen has been recovered but the disease has not been observed (San Diego County), an area in which the disease occurs infrequently and with mild severity (Santa Maria) but is adjacent to an area in which the disease is rampant (Oceano), and an area in which the disease is becoming a serious problem (Salinas). Soils from each site were placed in 15-cm-diameter pots and celery seedlings (one per pot) were transplanted therein. After plants were grown in the greenhouse for 4 wk, the roots were sieved from the soil, surface sterilized, and quantitatively assayed for infection by *F. oxysporum*. Colonies were randomly selected from the plates, single-spored, and cultures were saved for further study.

Inocula of each test isolate and *ora-3* were incorporated into the greenhouse soil at 0.5 g/kg and dispensed into four 7.5-cm-diameter pots. Two other pots received the test isolates alone. Inoculum consisted of colonized bits of barley straw as described above. Two controls, four pots each, were included with each assay, one received *ora-3* alone and the other remained uninoculated. Sterilized but uninoculated barley straw was added to the soils that did not receive inocula of both a test isolate and *ora-3* such that each soil contained a total of 1.0 g of barley straw per kilogram of soil. Plants (one per pot) were grown for 4 wk, the roots were sieved from the soil, surface sterilized, and assayed for infection by *ora-3*. Pathogenicity of the test isolates was assessed after 8 wk in the two pots inoculated with the test isolates alone. In a separate experiment, there was no statistically significant difference ($P = 0.05$) in rate of root infection between plants grown in soils infested with 0.5 g *ora-3* inoculum per kilogram of soil and soils either unamended or amended with up to 6.0 g of sterilized barley straw per kilogram (*unpublished*). Shoot dry weights were determined for all plants. Because all 228 isolates could not be tested at one time, root infection data are presented as a proportion of the control (*ora-3* alone) such that a value for relative root infection of <1.0 indicates that the test isolate suppressed infection by *ora-3* while a value >1.0 indicates enhancement. Root infection by *ora-3* alone ranged from 4.6 to 8.0 colonies per 100 cm in the four tests.

In another test, the most effective (suppressive) isolates from the S soil in Ventura County and Santa Maria, ineffective isolates from San Diego, and race 1 and 2 isolates (23) were evaluated for suppressive ability as described above except that various relative IDs also were included. Soil was amended with 0.5 g of *ora-3* inoculum per kilogram and 0.05, 0.10, 0.50, 1.0, or 5.0 g of inoculum of each test isolate per kilogram. This resulted in IDs of the test isolates relative to *ora-3* of 0.1, 0.2, 1.0, 2.0, and 10.0. As before, sterilized barley straw was added so that each soil contained a total of 5.5 g of barley straw per kilogram of soil. Rates of root infection were compared to the control, which received inoculum of *ora-3* alone. Additional controls were included that consisted of plants grown in soil amended with the various inoculum levels of each of the test isolates alone. Shoot dry weights were determined. There were eight replications and the experiment was repeated once with similar results.

Barley straw inocula of each of two isolates that suppressed (VS-11 and VS-15) or that enhanced (NY-145 and SD-80) root infection by *ora-3* were prepared as described above. The soil mix was amended with 1.0 g/kg of inoculum of each of the test isolates and each was divided into seven equal portions. One portion was amended with *ora-3* inoculum at each of the following rates: 0, 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 g/kg of soil. Sterilized barley straw was added so that each soil contained a total of 5.0 g of barley straw per kilogram of soil.

Because plant water potential (ψ_{plant}) affects root infection (*unpublished*), these experiments were conducted in soil tension plates, similar to those described by Duniway (7), so that soil water potential (ψ_{soil}) could be controlled. Briefly, the stems of 7-cm-diameter Büchner funnels with fritted glass plates of fine porosity were fitted with rubber stoppers and a short length of flexible plastic tubing. The funnels were then inverted, and the stems and tubing were filled with degassed water. Each funnel was then attached to a 1,000-ml side-arm vacuum flask containing 500 ml of degassed water taking care that no air entered the stem and tubing. The top portion of each funnel was immediately filled with a saturated experimental soil. The side arm of each flask was connected to a manifold with a constant suction maintained by a hanging column of water that generated a matric ψ_{soil} of -200 mb.

After the soil in the tension plates had equilibrated ($\sim$30 min), one celery seedling was transplanted into each funnel. Plants were fertilized twice weekly with half-strength Hoagland's solution (11) and illuminated (10 hr/day) by sodium and mercury vapor lamps (Environmental Growth Chambers, Chagrin Falls, OH 44022) and four cool-white fluorescent lamps that provided photosynthetically active radiation of 900 $\mu E \cdot m^{-2} \cdot s^{-1}$ at plant height. The constant suction was applied to the tension plates during the day and released at night. This resulted in ψ_{plant} of -6.5 bars at midday and -0.8 bars at midnight as measured with an isopiestic thermocouple psychrometer. Air temperature varied between 22 and 26 C.

After 3 wk, the roots were removed from the soil, surface sterilized, and quantitatively assayed for infection by *ora-3* as described above. Because of the limited number of tension plates, there was one replication per treatment, but the experiment was repeated three times. Data from all four repetitions were combined for statistical analyses.

Root infection as a function of time after transplanting. An experiment was conducted to establish the relationship between root infection and time after transplanting in an attempt to determine if infection occurred continually or was confined to a relatively short period of time. Soil was amended with *ora-3* at 0.5 g inoculum per kilogram of soil, dispensed into tension plates, and celery seedlings (one per plate) were transplanted therein. The plants were grown as described above and the roots from separate plants were assayed for root infection at 2, 3, 4, and 5 wk after transplanting. There were five replications and the experiment was repeated once with similar results.

Biological control. Barley straw inoculum of isolate 40-1, a highly virulent isolate, was incorporated into the greenhouse soil mix at 1.0 g/kg. Portions of this soil were each amended with inoculum of isolates VS-11, VS-15, SM-8, SM-9, SD-54, SD-78, and SD-80 each at the rates of 0.0, 0.1, 0.5, 1.0, and 2.0 g/kg of soil. In addition, inoculum of each of the test isolates was incorporated into soil (1.0 g/kg) without the pathogen. Each of the amended soils was dispensed into ten 15-cm-diameter pots, and celery seedlings (one per pot) were transplanted therein. An unamended control also was included. The plants were grown for 8 wk and rated for disease severity as before. Shoot dry weights also were recorded. The experiment was repeated once with similar results.

Soil was collected from a celery field that was severely affected by the disease, air-dried, and ground to pass through a 0.4-cm mesh. Pathogen ID was 187 ($\pm$53) propagules per gram. Barley straw inoculum of each test isolate (VS-11, VS-15, SM-8, and SM-9) was mixed with the soil at 2 g/kg. The control received sterilized barley straw at the same rate of addition. The amended soils were dispensed into 20-cm-diameter pots (10 pots per isolate) and seedlings were transplanted therein (one per pot). Plants were grown as described above and assessed for disease severity and dry

weight after 6 wk. Portions of the experiment were repeated once with similar results.

RESULTS

Demonstration of disease suppression. There was a linear decline in disease severity as the proportion of nonsterile S soil was increased with mild symptoms occurring in undiluted S soil (Fig. 2). When the same dilutions were made with sterilized S soil, there were no significant effects until the proportion of S soil reached 0.9. The slope of the first portion of the regression is not significantly different from zero ($P = 0.10$). Even though regression analysis of the last three points did not result in a significant linear relationship ($r = 0.958$, $P = 0.05$), the slope of the line is clearly different from that of the first six points. Essentially the same results were obtained when the experiment was repeated.

Rates of root infection. The effects of time exposure to the sterilant, NaOCl, on numbers of colonies of *F. oxysporum* per 100 cm of root were similar for roots collected from the suppressive and conducive sites with the exception of 0 min (Fig. 3). At zero time, there were 204 and 67 colonies per 100 cm in roots collected from the S and C soils, respectively, about a threefold difference. There were no significant differences in root infection for any of the other treatment periods. Because the 4-min treatment had the smallest standard error (Fig. 3), this time of exposure was used in all subsequent assays. Although there was no significant difference in rates of root infection by total *Fusarium* spp. (almost exclusively *F. oxysporum*) between S and C soils at the 4-min exposure, discrimination on the basis of pathogenicity indicated that roots collected from the suppressive sites were infected by pathogenic and nonpathogenic isolates at the rates of 2.6 and 15.9 colonies per 100 cm, respectively, while roots from the conducive sites had 10.4 and 2.8 colonies per 100 cm, respectively. This represents a near-perfect reciprocal relationship. A total of 243 pathogenicity tests were conducted in this assay. Similar results were obtained in another assay of roots from the same soils in which colony morphology (29) was used as the sole criterion for enumerating pathogenic isolates (*unpublished*).

The tedious and time-consuming procedures used in estimating root infection by the pathogen greatly limited the extent of additional studies. However, the greenhouse test that was devised using *ora-3* greatly simplified the assays and made possible a reliable means of measuring root infection by the pathogen without extensive subculturing and pathogenicity tests. Incorporation of 0.5 g of inoculum per kilogram of soil, used in subsequent experiments, resulted in approximately half (8.2 infections per 100 cm of root) the maximum rate of root infection (16.1 and 17.4 infections per 100 cm of root) obtained at 2.0 and 5.0 g/kg, respectively. By using this level of root infection as a reference, both enhancement and suppression of root infection could be assessed. The standard rate of incorporation was equivalent to a population

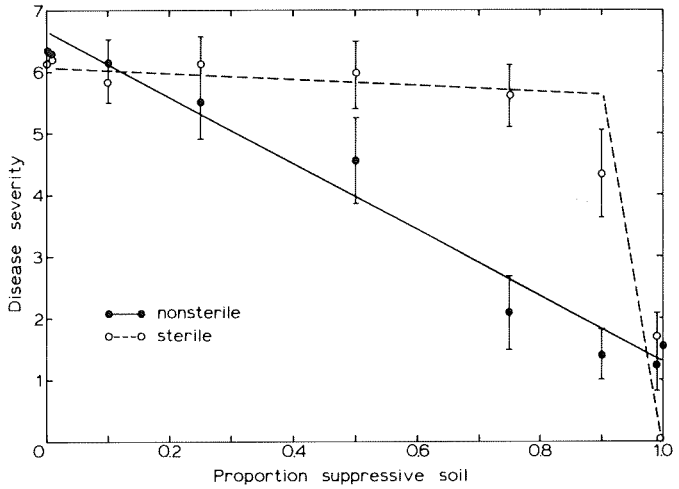

Fig. 2. Effect of different proportions of suppressive and conducive soil (S and C soil, respectively) on severity of Fusarium yellows in celery. S soil was either sterilized or unsterilized before being mixed with the C soil. Vertical bars indicate ± standard error.

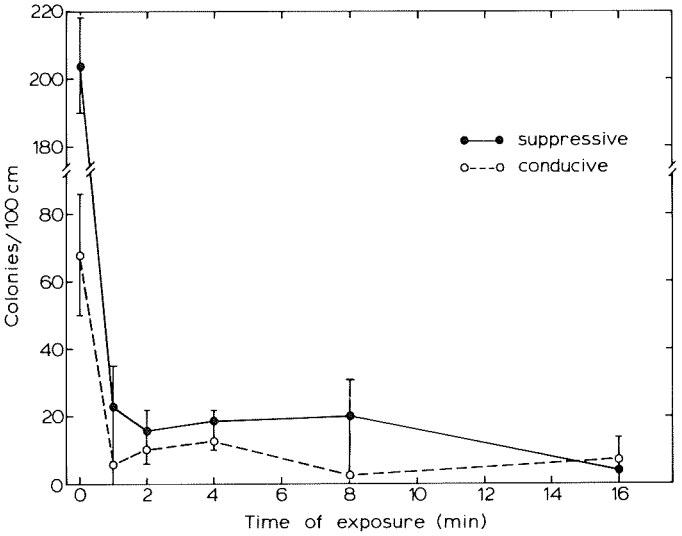

Fig. 3. Influence of time of exposure of celery roots to 0.21% sodium hypochlorite on apparent root infection (colonies per 100 cm of root) by *Fusarium oxysporum*. Roots were collected from areas within a single field that were either suppressive or conducive to Fusarium yellows of celery (See Fig. 1). Vertical bars indicate ± standard error.

TABLE 2. Infection of celery roots by an orange-colored mutant (*ora-3*) of *Fusarium oxysporum* f. sp. *apii* as affected by isolates of *F. oxysporum* collected from surface-sterilized roots of celery plants growing in disease suppressive (S) and conducive (C) areas

Location and designation	Nonpathogenic[a] Isolates (no.)	Nonpathogenic[a] Relative root infection[b]	Nonpathogenic[a] S.E.[c]	Pathogenic[a] Isolates (no.)	Pathogenic[a] Relative root infection	Pathogenic[a] S. E.	Colonies per 100 cm root[d]
Ventura (S)	36	0.46	0.22	21	0.73	0.30	18.5
Ventura (C)	27	1.01	0.40	26	0.76	0.34	13.2
Oceano	6	1.07	0.26	22	1.08	0.86	14.1
Santa Maria	24	0.39	0.29	7	0.71	0.27	19.0
Salinas	18	1.49	0.42	15	0.93	0.49	15.4
San Diego	24	0.99	0.58	2	0.84	0.22	16.7
LSD[e]		0.45			N.S.[f]		N.S.

[a] Isolates were either pathogenic or nonpathogenic when tested by themselves against celery.
[b] Root infection is presented as a proportion of that observed for plants grown in the presence of *ora-3* alone.
[c] Standard error.
[d] Rate of root infection by *F. oxysporum* of celery plants growing at the indicated location.
[e] Least significant difference ($P = 0.05$).
[f] Not significantly different ($P = 0.05$) according to an analysis of variance.

of *ora-3* of about 270 propagules per gram of soil as determined by dilution plating on Komada's medium.

Effects of pathogenic and nonpathogenic isolates of *F. oxysporum* on root infection by *ora-3*. There were significant differences in relative infection by *ora-3* as affected by nonpathogenic isolates of *F. oxysporum* collected from the S and C soils (Table 2). Values for the S soils were approximately half those of the C soils. There was a wide range in response to the pathogenic isolates with no statistically significant differences among sampling sites (Table 2).

Comparisons of isolates from the four other locations indicated that there was a wide range in effects of nonpathogenic Fusaria on infection by *ora-3* (Table 2). The population of nonpathogenic *F. oxysporum* sampled from Santa Maria was significantly more effective in reducing infection by *ora-3*, while the population from Salinas was significantly less effective (Table 2). There were no statistically significant differences among pathogenic isolates in their ability to affect infection by *ora-3* from any of the locations.

There was a significant inverse linear correlation (r = 0.604, degrees of freedom = 225, and P = 0.01) between plant dry weight and relative infection by *ora-3* as affected by the test isolates. The regression equation is $Y = -0.465\,X + 1.736$ in which Y is relative dry weight and X is relative root infection. There were no significant differences in dry weight between plants inoculated with any of the nonpathogenic test isolates alone and the uninoculated controls.

There were significant differences among test isolates at all inoculum levels in affecting infection by *ora-3* (Table 3). Two of the Race 1 isolates (nos. 60 and NY-145) and the race 2 isolate (no. 40) enhanced infection by *ora-3*, while 18142 (race 1) significantly repressed infection at a relative inoculum density of 1.0. Clearly, there were significant differences among the pathogenic isolates in affecting infection by *ora-3* at all inoculum levels. Isolates that were suppressive in the previous test (VS-11, VS-15, SM-8, and SM-9) were consistent in their behavior. However, they were most effective at relative inoculum densities of 1.0–2.0. The San Diego isolates (SD-54, SD-78, and SD-80) were relatively ineffective or enhanced infection by *ora-3*. As in the previous study, there was a significant inverse linear correlation between rate of root infection by *ora-3* and dry weight of plants (r = 0.58, degrees of freedom = 53, and P = 0.01).

TABLE 3. Infection of celery roots by an orange mutant (*ora-3*) of *Fusarium oxysporum* f. sp. *apii* (*Foa*) as affected by nonpathogenic isolates of *F. oxysporum* and race 1 and race 2 isolates of *Foa* at several inoculum levels

Test isolates	Relative infection of celery roots by *ora-3* in the presence of test isolates at inoculum level[a]: 0.1	0.2	1.0	2.0	10.0	LSD[b]
40[c]	1.26[d]	1.40	2.35	3.02	2.67	1.58
60[e]	1.74	1.21	0.87	5.84	3.55	1.33
NY-145[e]	2.34	2.24	1.55	1.37	0.63	1.21
18142[e]	0.89	0.45	0.37	0.58	1.32	0.64
VS-11[f]	1.11	1.05	0.42	0.48	0.89	0.39
VS-15[f]	1.20	0.91	0.38	0.36	0.97	0.32
SM-8[g]	1.18	1.29	0.61	0.37	0.39	0.47
SM-9[g]	1.07	1.10	0.54	0.49	0.61	0.52
SD-54[h]	1.68	1.82	1.42	1.21	0.76	1.11
SD-78[h]	1.30	1.00	0.94	0.87	0.89	0.76
SD-80[h]	2.55	1.74	1.81	1.94	1.89	0.98
LSD	0.57	0.50	0.48	0.61	0.84	

[a] Inoculum levels of the test isolates are relative to that of *ora-3*, which was 0.5 g/kg soil.
[b] Least significant difference (P = 0.05).
[c] Race 2 of *Foa* (23).
[d] Root infection is presented as a proportion of that observed for plants grown in the presence of *ora-3* alone.
[e] Race 1 of *Foa* (23).
[f] Suppressive isolates. See Table 2.
[g] Isolates from Santa Maria. See Table 2.
[h] Isolates from San Diego. See Table 2.

Quantifying the interaction between inoculum density of *ora-3* and the effect of suppressive and conducive isolates. The maximum rate of root infection by *ora-3* (20.5 to 22.5 colonies per 100 cm) in the absence of other isolates occurred at an ID of about 2.0 g of inoculum per kilogram of soil (Fig. 4). In the presence of either of the two suppressive isolates (VS-11 and VS-15), the amount of *ora-3* inoculum required to cause the maximum rate of root infection was doubled. The addition of either conducive isolate (NY-145 or SD-80) resulted in enhanced infection at the lower IDs and a higher maximum rate of infection at the higher IDs relative to inoculation with *ora-3* alone. The effects of other isolates on infection by *ora-3* are difficult to interpret in Fig. 4 because both the maximum rate of root infection and the ID at which this rate was attained varied among the isolates. Similar difficulties are encountered in enzyme kinetics in which the roles of inhibitors must be quantitatively assessed (26). The use of the Lineweaver-Burk double reciprocal plot technique (26) greatly facilitates enzymatic analyses by providing mathematically defined values for comparative purposes. This approach was used in the present study as described below.

In biochemical studies the reciprocal of enzyme velocity ($1/v$) is plotted as a function of the reciprocal of substrate concentration ($1/S$). Provided that the nontransformed relationship is hyperbolic, such a plot yields a straight line such that the slope is K_m/V_{max}, the intercept of the $1/v$ axis is $1/V_{max}$, and the intercept of the $1/S$ axis is $-1/K_m$, where V_{max} is maximum reaction velocity and K_m is the Michaelis-Menten constant or $V_{max}/2$. These two values, V_{max} and K_m, provide a mathematically defined means for analyzing reaction kinetics.

In the present analogy, grams of inoculum per kilogram of soil (I), colonies per 100 cm of root (C), and C_{max} were substituted for S, v, and V_{max}, respectively. In addition, the value for K_m represents the I required for half the extrapolated maximum rate of root infection. The linear relationships of the reciprocals of I versus C were calculated (Table 4) and plotted (Fig. 5). Values for K_m (negative reciprocal of the $1/I$ intercept) (Fig. 5) and C_{max} (reciprocal of the $1/C$ intercept) (Fig. 5) were calculated for each isolate (Table 4). Thus, 1.06 g of inoculum of *ora-3* per kilogram of soil in the absence of other isolates were required to cause half the maximum rate of root infection (28.6 colonies per 100 cm). The inclusion of VS-15 increased the K_m to 3.94 g/kg, while SD-80 reduced the K_m to 0.24 and increased the C_{max} to 34.6 colonies per 100 cm. The coefficients of variation for K_m and C_{max} for the five isolates were 94.9 and 23.0%, respectively.

The hyperbolic nature of the curves in Fig. 4 was tested as described by Segel (26). Inasmuch as the curvatures of hyperbolae are fixed regardless of such arbitrarily chosen values as K_m and C_{max}, the ratios of I required for any two fractions of C_{max} should be constant. In this case the ratios of I required for 90 and 10% of C_{max}

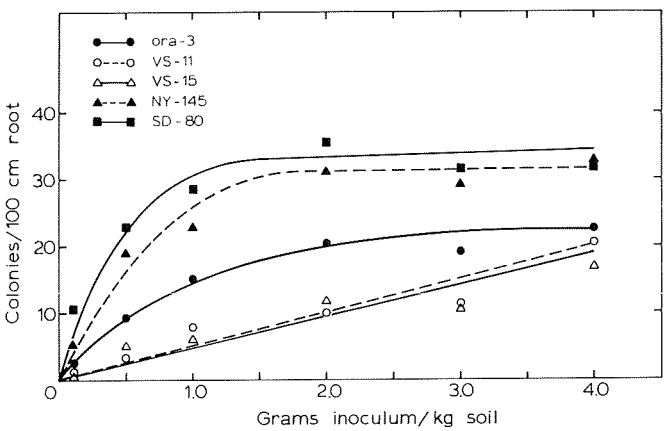

Fig. 4. Relationships between inoculum density (grams of inoculum per kilogram of soil) of an orange mutant (*ora-3*) of *Fusarium oxysporum* f. sp. *apii* and rate of celery root infection (colonies per 100 cm of root) as affected by coinoculation (1.0 g/kg) with several nonpathogenic isolates of *F. oxysporum*.

were calculated for each isolate. The ratios are 80.1, 82.3, 80.5, 79.9, and 75.0 for *ora-3* alone, VS-11, VS-15, NY-145, and SD-80, respectively. Because there are no statistically significant differences ($P = 0.10$) among the ratios, the curves in Fig. 4 are considered to be hyperbolic.

Root infection as a function of time after transplanting. Rates of root infection for plants sampled at 2, 3, 4, and 5 wk after transplanting were 8.6, 8.0, 7.9, and 8.5 colonies per 100 cm of root, respectively. There were no statistically significant differences ($P = 0.10$) among the values according to an analysis of variance.

Biological control. Coinoculation of soils with the pathogen and either isolate VS-11 or VS-15 resulted in a substantial reduction in disease severity (Table 5) that was reflected by increases in plant dry weight (Table 6). The most pronounced effect occurred following the addition of 0.5–1.0 g of inoculum per kilogram of soil. There was very little difference between 1.0 and 2.0 g/kg. Isolate SD-80 accentuated disease severity and significantly reduced plant growth relative to the pathogen alone. The regression equation, relating relative dry weight (RDW) and disease severity index (DSI) is RDW = 1.11 − 0.17 DSI ($r^2 = 0.87$, significant at $P = 0.01$).

DSI and dry weight were significantly affected following the incorporation of the test isolates into the raw field soil. DSI was 2.9, 2.6, 3.7, and 4.3 (LSD = 0.9, $P = 0.05$) while dry weights were 5.6, 6.0, 5.2, and 4.3 g (LSD = 1.1, $P = 0.05$) for isolates VS-11, VS-15, SM-8, and SM-9, respectively. DSI and dry weight in the nonamended control were 6.2 and 1.2 g, respectively.

DISCUSSION

Inasmuch as there were no significant differences in ID of the pathogen or soil chemical characteristics known to affect disease development (8,20) between the suppressive and conducive areas within the one field, it is proposed that the small areas of healthy plants in the otherwise uniformly devastated field are an example of disease suppression sensu Baker and Cook (3). Furthermore, the different trends in disease severity as a function of proportion of suppressive soil between the sterilized and unsterilized treatments implicate a biological agent of suppression. The lack of response (slope not significantly different from zero) to a 10-fold dilution of the conducive soil with sterilized suppressive soil is not surprising in light of the fact that the ID of the field soil was >200 propagules per gram, and IDs of <20 propagules per gram cause 100% disease (29). The steep decline in disease severity with sterile soil dilutions of >10-fold probably reflects a reduction in ID below that required for 100% disease. In contrast, the linear reduction in disease severity over the entire range of dilutions with nonsterile soil implicates a biological factor in disease suppression that did not multiply during the 10-day equilibration period.

These results are in contrast to other cases of disease suppression in which very small proportions of S soil confer a high level of disease control (4,5,25). A significant reduction in disease severity following the addition of 1% or less of S soil to C soil (5,27) implies that the suppressive agent multiplied during the period of observation, while the linear response obtained in the present study indicates that there was no multiplication. Rhizosphere bacteria with extremely short generation times have been implicated in the former case, with the take-all decline phenomenon (5,12,30) and a soil suppressive to several Fusarium wilts (12) as prime examples. The latter case, ie, no multiplication, is exemplified by suppression of *Rhizoctonia solani* (31), Fusarium wilt of muskmelon (1,2,18), and take-all of wheat (6,33), all of which may be caused by antagonistic or competitive fungi. Interestingly, the relationship

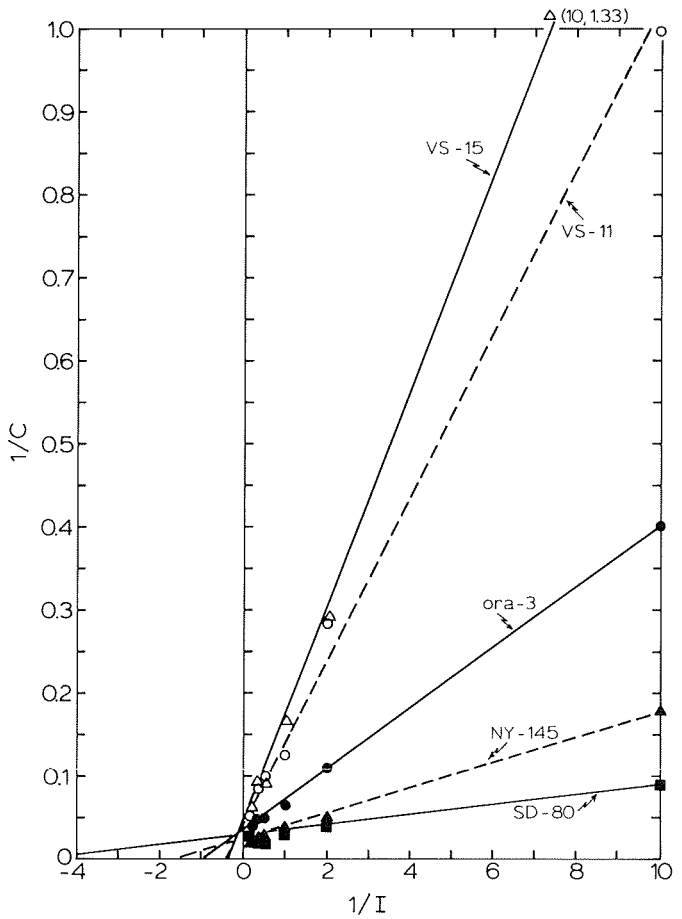

Fig. 5. Relationships between the reciprocal of inoculum density ($1/I$) (grams of inoculum per kilogram of soil) of an orange mutant (*ora-3*) of *Fusarium oxysporum* f. sp. *apii* and the reciprocal of the rate of root infection ($1/C$) (colonies per 100 cm of root) as affected by coinoculation (1.0 g/kg) with several nonpathogenic isolates of *F. oxysporum*.

TABLE 4. Components of the linear relationships of the reciprocals of grams of inoculum (I) of an orange mutant (*ora-3*) of *Fusarium oxysporum* f. sp. *apii* (*Foa*) per kilogram of soil versus the rate of celery root infection (colonies per 100 cm of root) (C) as affected by various test isolates. See Fig. 5 for a graphical representation

Isolates	Slope	Intercepts		K_m[u]	C_{max}[v]
		$1/I$	$1/C$		
Control[w]	0.037 a[x]	−0.946 b	0.035 b	1.06 b	28.6 b
VS-11[y]	0.096 b	−0.521 a	0.050 c	1.92 c	20.0 a
VS-15[y]	0.130 c	−0.254 a	0.033 ab	3.94 d	30.3 b
NY-145[z]	0.016 d	−1.625 c	0.026 a	0.61 ab	38.5 c
SD-80[y]	0.007 e	−4.143 d	0.029 ab	0.24 a	34.6 bc

[u] Negative reciprocal of the $1/I$ intercept.
[v] Reciprocal of the $1/C$ intercept.
[w] *ora-3* alone.
[x] Values followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[y] Nonpathogenic isolates of *F. oxysporum*.
[z] Race 1 of *Foa*.

TABLE 5. Effect of amending soil with *Fusarium oxysporum* f. sp. *apii* and various amounts of inoculum of nonpathogenic isolates of *F. oxysporum* on the disease severity index (DSI)[a]

Nonpathogenic isolates	DSI at inoculum level (g/kg soil)[b]:					LSD[c]
	0	0.1	0.5	1.0	2.0	
VS-11	5.7	4.6	3.7	2.6	2.5	1.1
VS-15	6.1	5.0	3.4	2.6	2.2	1.1
SM-8	6.0	5.3	4.0	2.5	3.0	0.9
SM-9	5.5	5.7	3.6	3.9	3.1	1.0
SD-54	5.5	6.0	4.5	4.8	4.7	0.8
SD-78	5.8	5.6	5.4	5.0	4.7	0.8
SD-80	6.2	6.0	6.6	6.9	7.0	0.6
LSD	1.0	1.0	0.8	1.3	1.3	···

[a] The experimental soil was amended with 1.0 g of barley straw inoculum of the pathogen per kilogram of soil.
[b] 1.0 = symptomless; 7 = wilted or dead.
[c] Least significant difference ($P = 0.05$).

between proportion of nonsterilized S soil and disease severity in the present study is essentially identical to that reported by Louvet et al (15) for suppression of Fusarium wilt of muskmelon, which has been associated with saprophytic strains of *F. oxysporum* (1,18).

The large differences in capacity to suppress or enhance root infection by *Foa* among populations of nonpathogenic *F. oxysporum* isolated from celery roots from suppressive and conducive areas within one field and other areas within the state strongly implicate these parasites in the observed phenomenon. The criteria for demonstrating suppressiveness were fulfilled in that the pathogen was recovered from roots of susceptible celery cultivars collected from areas in which the disease did not occur. In addition, there were no apparent differences in effects on root infection by *ora-3* among pathogenic isolates from any location. Furthermore, the ability of selected isolates to suppress disease in a raw soil collected from a severely diseased field demonstrates that they are capable of functioning in the natural soil milieu.

Effective competitors are defined as those isolates that infect the root and suppress infection by the pathogen. Ineffective competitors are isolates that can infect but do not suppress infection by the pathogen. Isolates that can be cultured from surface-sterilized roots are considered to be capable of infection. Inasmuch as the test isolates, including the pathogens, were obtained from surface-sterilized, symptomless roots, it is concluded that all the isolates used in this study are capable of root infection.

The lack of differences in rate of root infection as a function of time after transplanting and the plateau in root infection with increasing ID suggest that there are a finite number of infection sites per unit length of root or that the sites of infection are susceptible for a finite time period. Root tips are known to be the sites of infection by pathogenic forms of *F. oxysporum* for several suscepts (14,16) including celery (10). Thus, it is reasonable to conclude that if these sites are occupied by effective nonpathogenic competitors, the rate of root infection by the pathogen would be reduced as a function of the ratio of pathogenic to competitive nonpathogenic IDs of *F. oxysporum*. Such was the case in the present study, except that at the highest ID ratio (10:1) of effective competitor to pathogen there was a stimulation of infection by *ora-3*. Thus, there appears to be a balance between effective competition and induced susceptibility or predisposition. Clearly, infection by race 1 of *Foa* and several nonpathogenic isolates predisposed the roots to infection by *ora-3*.

The use of K_m and C_{max} as derived by the Lineweaver-Burk technique in this study are significant in that these mathematically defined values provide a means for quantifying the effects of competitive infection. The large differences in slopes in the reciprocal plots are caused by factors that affect either or both of the derived values. Thus, the test isolates affected either the effectiveness of the *ora-3* inoculum or the maximum number of susceptible sites per unit length of root or both. For example, the differences in K_m values among *ora-3* alone, VS-15, and SD-80 suggest that VS-15 reduced the effectiveness of the *ora-3* inoculum by 3.7-fold, while SD-80 increased its effectiveness by 4.4-fold. The significantly larger coefficient of variation for K_m compared to C_{max} suggests that the primary means by which the test isolates either enhanced or suppressed infection by *ora-3* was by rendering the finite number of infection sites more susceptible, rather than by increasing the number of such sites. Biological mechanisms cannot be inferred from these analyses, but the roles of these isolates in inducing defense measures and in affecting membrane leakage and intercellular concentrations of soluble substrates warrant further study.

Assuming that the relationships between ID and root infection or disease incidence are hyperbolic for other host-pathogen systems, calculations of K_m and C_{max} would provide useful information in addition to that described in the present study. The influence of environmental factors, such as soil moisture, fertility, and oxygen status, on inoculum effectiveness could be assessed on a quantitative basis. Also, host plant resistance could be determined with precision.

The suppression of Fusarium yellows of celery by nonpathogenic strains of *F. oxysporum* as described in the present study is remarkably similar to the findings of Alabouvette et al (1) and Rouxel et al (18) who related suppression of Fusarium wilt of muskmelon specifically to populations of nonpathogenic *F. oxysporum*. The primary difference is in the role ascribed to the nonpathogenic strains. Alabouvette et al (1,2) concluded that the competition that results in suppression is saprophytic in nature and occurs in niches in the soil apart from the suscept. Although saprophytic competition cannot be excluded in the present study, results presented herein suggest that parasitic competition is the primary mechanism of suppression. Similar conclusions regarding avirulent or weakly virulent parasites were reported for take-all of wheat and turf (6,32,33), the primary difference being that disease control by these organisms does not appear to function in disease-suppressive wheat soils (6), and its role in suppressive turf soil is questionable (32).

The potential for exploiting nonpathogenic competitive strains of *F. oxysporum* as biological control agents in the field is unknown. However, the ecological and physiological similarities between pathogenic and nonpathogenic strains of this species should prove to be an asset in that both parasitic and saprophytic competition could be employed. Areas to be investigated in this regard include the role of nonsuscepts as rotation crops in affecting relative populations of the pathogen and competitors and determining the feasibility of introducing and maintaining high populations of selected competitors. The use of pigmented mutants and the Lineweaver-Burk plots, as described in the present study, will be helpful in such studies.

TABLE 6. Effect of amending soil with *Fusarium oxysporum* f. sp. *apii* and various amounts of inoculum of nonpathogenic isolates of *F. oxysporum* on relative dry weights (RDW) of celery shoots[a]

Nonpathogenic isolates	RDW at inoculum level (g/kg soil)[b]: 0	0.1	0.5	1.0	2.0	RDW with nonpathogenic isolate alone[c]	Dry weight of unamended control (g)	LSD[d]
VS-11	0.18	0.26	0.48	0.71	0.79	1.11	6.6	0.11
VS-15	0.10	0.21	0.53	0.75	0.71	1.07	6.7	0.13
SM-8	0.12	0.23	0.39	0.80	0.82	1.10	7.0	0.15
SM-9	0.15	0.19	0.30	0.56	0.63	0.95	6.3	0.09
SD-54	0.12	0.14	0.20	0.18	0.26	0.92	6.1	0.15
SD-78	0.16	0.19	0.19	0.23	0.20	0.94	6.5	0.11
SD-80	0.15	0.11	0.10	0.09	0.03	0.97	6.0	0.04
LSD[d]	0.07	0.10	0.13	0.09	0.15	0.10	0.3	...

[a] Soil was amended with 1.0 g of barley straw inoculum of the pathogen per kilogram of soil.
[b] Shoot dry weight as a proportion of the unamended control.
[c] Incorporated at the rate of 1.0 g of inoculum per kilogram of soil.
[d] Least significant difference ($P = 0.05$).

LITERATURE CITED

1. Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of Fusarium wilt-suppressive soils and prospects for their utilization in biological control. Pages 165-182 in: Soil-Borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, New York. 686 pp.
2. Alabouvette, C., Rouxel, F., and Louvet, J. 1980. Recherches sur la résistance des sols aux maladies VI.—Mise en évidence de la spécificité del la résistance d'un sol vis-à-vis des Fusariouses vasculaires. Ann. Phytopathol. 12:11-19.
3. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman, San Francisco. 433 pp.
4. Cook, R. J. 1982. Use of pathogen-suppressive soils for disease control. Pages 51-65 in: Suppressive Soils and Plant Disease. R. W. Schneider, ed. Am. Phytopathol. Soc., St. Paul, MN. 88 pp.
5. Cook, R. J., and Rovira, A. D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol. Biochem. 8:269-273.
6. Deacon, J. W. 1976. Biological control of the take-all fungus, *Gaeumannomyces graminis*, by *Phialophora radicicola* and similar fungi. Soil Biol. Biochem. 8:275-283.
7. Duniway, J. M. 1975. Formation of sporangia by *Phytophthora drechsleri* in soil at high matric potentials. Can. J. Bot. 53:1270-1275.
8. Hart, L. P. 1978. The etiology and biology of Fusarium yellows of celery. Ph.D. dissertation. University of California, Riverside. 148 pp.
9. Hart, L. P., and Endo, R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Rep. 62:138-142.
10. Hart, L. P., and Endo, R. M. 1981. The effect of time of exposure to inoculum, plant age, root development, and root wounding on Fusarium yellows of celery. Phytopathology 71:77-79.
11. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 39 pp.
12. Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. 1980. *Pseudomonas* siderophores: A mechanism explaining disease-suppressive soils. Curr. Microbiol. 4:317-320.
13. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.
14. Locke, T., and Colhoun, J. 1977. A process of infection of oil palm seedlings by *Fusarium oxysporum* f. sp. *elaeidis*. Phytopathology 67:18-28.
15. Louvet, J., Alabouvette, C., and Rouxel, F. 1981. Microbiological suppressiveness of some soils to Fusarium wilts. Pages 261-275 in: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park. 457 pp.
16. Nyvall, R. F., and Haglund, W. A. 1976. The effect of plant age on severity of pea wilt caused by *Fusarium oxysporum* f. sp. *pisi* Race 5. Phytopathology 66:1093-1096.
17. Puhalla, J. E. 1984. A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. Can. J. Bot. 62: (In press).
18. Rouxel, F., Alabouvette, C., and Louvet, J. 1979. Recherches sur la résistance des sols aux maladies IV.—Mise en évidence du rôle des *Fusarium* autochtones dans la résistance d'un sol à la Fusariose vasculaire du melon. Ann. Phytopathol. 11:199-207.
19. Ryder, E. J. 1979. Leafy Salad Vegetables. AVI Publishing Co., Inc., Westport, CT. 266 pp.
20. Schneider, R. W. 1981. Influence of nitrate and potassium on severity of Fusarium yellows of celery. (Abstr.) Phytopathology 71:903.
21. Schneider, R. W., ed. 1982. Suppressive Soils and Plant Disease. Am. Phytopathol. Soc., St. Paul, MN. 88 pp.
22. Schneider, R. W. 1983. Suppression of Fusarium yellows of celery by competitive nonpathogenic strains of *Fusarium oxysporum*. (Abstr.) Phytopathology 83:814.
23. Schneider, R. W., and Norelli, J. L. 1984. A new race of *Fusarium oxysporum* f. sp. *apii* in California. Plant Dis. 68:(In press).
24. Schneider, R. W., and Pendery, W. E. 1983. Stalk rot of corn: Mechanism of predisposition by an early-season water stress. Phytopathology 73:863-871.
25. Schroth, M. N., and Hancock, J. G. 1982. Disease-suppressive soil and root-colonizing bacteria. Science 216:1376-1381.
26. Segel, I. H. 1976. Biochemical Calculations. John Wiley & Sons, New York. 441 pp.
27. Shipton, P. J., Cook, R. J., and Sitton, J. W. 1973. Occurrence and transfer of a biological factor in soil that suppresses take-all of wheat in eastern Washington. Phytopathology 63:511-517.
28. Toussoun, T. A., and Nelson, P. E. 1976. A Pictorial Guide to the Identification of *Fusarium* Species. Pennsylvania State University Press, University Park. 43 pp.
29. Welch, K. E. 1981. The effects of inoculum density and low oxygen tensions on Fusarium yellows of celery. Ph.D. dissertation. University of California, Berkeley. 130 pp.
30. Weller, D. M., and Cook, R. J. 1981. Pseudomonads from take-all conducive and suppressive soils. (Abstr.) Phytopathology 71:264.
31. Wijetunga, C., and Baker, R. 1979. Modeling of phenomena associated with soil suppressive to *Rhizoctonia solani*. Phytopathology 69:1287-1293.
32. Wong, P. T. W., and Siviour, T. R. 1979. Control of Ophiobolus patch in *Agrostis* turf using avirulent fungi and take-all suppressive soils in pot experiments. Ann. Appl. Biol. 92:191-197.
33. Wong, P. T. W., and Southwell, R. J. 1980. Field control of take-all of wheat by avirulent fungi. Ann. Appl. Biol. 94:41-49.