Evidence that Antagonistic Bacteria Suppress Fusarium Wilt of Celery in Neutral and Alkaline Soils

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ABSTRACT

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The severity of Fusarium yellows of celery was increased significantly as the pH of U.C. soil mix was reduced from 8.3 to 3.9. Chlamydospore germination of *Fusarium oxysporum* f. sp. *apii* in 0.1 *M* citrate buffer was maximal at pH 7.1, but no significant difference in germination occurred between pH 3.3 and 9.6. Dry weights of the fungus after 1 wk of growth in nutrient broth were maximal at pH 4.0 and 9.6. In cultures coinoculated with *Corynebacterium* sp., maximal growth of mycelial mats of *F. oxysporum* f. sp. *apii* occurred at pH 4.0; growth was significantly less at pH 6.2 and 7.3 than at 5.1. When the pathogen was grown in dual culture with each of 24 different bacterial isolates from four soil types, the number of bacterial isolates that did not allow significant mycelial growth increased from 16 to 54 and 75% as the pH of nutrient broth was increased from 5.1 to 6.2 and 7.3, respectively. When chlamydospores were germinated in buffered and pH-adjusted nutrient broth previously inoculated with *Corynebacterium* sp., germination after 2 days was significantly less than that of the sterile control at pH 5.1 or above. In a Noble agar medium adjusted to pH 5.7 or 6.9, the number of germ tubes originating from chlamydospores impinging on celery root tips was significantly greater in roots treated with streptomycin, as compared with roots treated with the active *Corynebacterium* culture.

Edgerton (2) was the first to demonstrate that soil pH influenced the amount and severity of infection of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Both disease incidence and severity were high at low soil pH and were low at neutral or slightly alkaline pH. Sherwood (19) and Jones and Woltz (6,7,9) verified these results, whereas others (8,15,18,20,23) demonstrated a similar relationship between soil pH and severity of Fusarium-incited diseases of bananas, cotton, wheat, cucumber, watermelon, and beans.

Jones and Woltz (6,7) and Woltz and Jones (24) investigated the basis of this relationship with the Fusarium wilt disease of tomato. They believe that liming Fusarium-infested field soil to pH 7.0–7.5 partially controls the disease because it limits the availability of microelements, zinc, copper, iron, manganese, and molybdenum, to the pathogen and that such microelements are required for growth, sporulation, and virulence of the fungus. Citing Waksman (22), Jones and Woltz (9) speculated that liming may also inhibit the development of Fusarium wilt because it favors the development of actinomycetes and bacterial populations that may suppress the development of fungal populations through the production of toxins and/or direct competition for organic and inorganic nutrients in the soil solutions.

This article presents data that indicate that the incidence and severity of Fusarium yellows disease of celery caused by *Fusarium oxysporum* f. sp. *apii* is also influenced by the pH of the soil and that part of this effect may be attributed to the differential effects of soil pH on the bacterial populations of the soil.

MATERIALS AND METHODS

Production of inoculum of the pathogen. *Fusarium oxysporum* Schlecht f. sp. *apii* (R. Nelson and Sherb.) Snyd. and Hans isolated from yellows-affected celery (*Apium graveolens* var. *dulce*) from Camarillo, CA, was used in this investigation. After single-sporing, the isolate was maintained in a tube of soil steamed twice before infestation with the fungus (21). When inoculum was desired, a few grains of infested soil were transferred to carnation-leaf water agar (21). After growth for 1 wk at 24 ± 2 C under continuous fluorescent illumination of 1,061 $\mu w \cdot cm^{-2}$ from Sylvania Gro-Lux® bulbs, mass transfers were made to increase inoculum. Inoculum consisting of a mixture of macroconidia and microconidia was produced on potato-dextrose agar (PDA) made from fresh potatoes and incubated for 2 wk at 24 ± 2 C under the continuous fluorescent illumination mentioned previously. A mixture of approximately 30% macroconidia and 70% microconidia was harvested by flooding each plate with 10 ml of sterile distilled water and passing the suspension through a double layer of cheesecloth to remove mycelial fragments.

Chlamydospores were produced by initially producing mycelial mats. A 5-mm agar plug of the fungus was taken from carnation-leaf water agar and transferred to 15 ml of V8-C broth held in a 9-cm petri plate. A thick mycelial mat resulted after incubation in the dark at 27 C for 5 days. The V8-C media was removed and replaced with 15 ml of sterile distilled water. After incubating mycelial mats in the dark for 2 wk at 27 C, abundant chlamydospore formation occurred. Chlamydospores were harvested by comminuting the mats in an ice-cooled cup for 5 min at maximum speed with a Sorvall Omni-mixer. The chlamydospores were then separated from the mycelial fragments by low-speed centrifugation for 10 sec, followed by removal of the supernatant. Three additional centrifugations usually separated chlamydospores from the mycelial fragments. Spore concentrations were determined by hemacytometer counts, and propagules were diluted with sterile distilled water before incorporation into steamed U.C. soil mix. When a natural form of inoculum was utilized, Fusarium yellows-affected celery crowns collected from a diseased celery field were used. Infected crown tissue (400 g) was comminuted in a blender containing sterile distilled water and brought to a total volume of 1,200 ml; to infest 1,834 g of steamed U.C. soil mix, 150 ml of this tissue was used.

Adjustment of pH in soil or agar media. The pH of steamed U.C. soil mix (peat moss and sand [1:1, v/v]) was adjusted by the addition of either KOH or H_2SO_4 (12 *M*) followed by thorough mixing (5). To measure the pH of soil, 10 g of soil mix was saturated with 0.01 *M* $CaCl_2$ and the pH determined with a Beckman® pH meter (17). Soil prepared in this way remained at the adjusted pH for 2 wk, which was long enough for germination and infection to occur (3,4). In another experiment, a constant pH was imposed by placing pots (10 × 10 × 10 cm) of chlamydospore-infested U.C. soil

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mix in shallow trays containing buffered and pH-adjusted Hoagland's solution (12). The Hoagland's solution was amended with KH_2PO_4 to give a 0.1*M* concentration, and the buffered solution was adjusted to different pH values with 12*M* KOH or H_2SO_4. The pH of the infested soil mix was determined using a surface electrode at weekly intervals after equilibration.

The effect of pH on mycelial growth of *F. oxysporum* f. sp. *apii* was investigated using oatmeal agar adjusted with either dilute KOH or H_2SO_4. The pH of the solidified medium was monitored using a Beckman® surface electrode at the initiation and termination of the experiment; no significant change in pH was noticed at the end of the experiment. The effect of pH on mycelial growth of *F. oxysporum* f. sp. *apii* was also investigated utilizing nutrient broth buffered with 0.5 g of KH_2PO_4/L and adjusted to various pH values with either dilute KOH or H_2SO_4 (1). When Noble agar was used, it was buffered with 0.1*M* sodium citrate and adjusted to pH 4.5, 5.7, and 6.9 with dilute KOH or H_2SO_4 (1).

Isolation and culture of bacteria used as coinoculants with *F. oxysporum* f. sp. *apii*. Bacteria grown in dual cultures with *F. oxysporum* f. sp. *apii* were isolated from U.C. soil mix and from three fresh soils (Chino silty clay loam, Hueneme sandy loam, and Pacheco silty clay loam) used for commercial celery production. Isolations were made from soil dilutions on tryptic soy agar (11). Six bacterial colonies with different characteristics were selected from the dilution plates of each soil type and streaked on nutrient agar. Individual colonies of each type were then transferred to nutrient agar slants for storage. One bacteria isolate used extensively in coinoculation experiments was isolated from U.C. soil mix and characterized as a *Corynebacterium* sp. Bacterial suspensions were prepared by adding sterile distilled water (10 ml) to a 1-wk-old culture of bacterium grown on a nutrient agar slant and mixing with a Vortex mixer. *F. oxysporum* f. sp. *apii* alone or combined with one of 24 different bacterial isolates was introduced into 125-ml flasks containing buffered and pH-adjusted nutrient broth by addition of a 5-mm plug of the fungus taken from a 1-wk-old PDA culture or three drops of a bacterial suspension. Cultures were incubated in triplicate for varying periods of time in the dark at 24 ± 2 C on a laboratory bench without agitation.

Measurements of direct effects of pH with and without bacteria on pathogen activity. To assess the effect of pH on the mycelial growth of *F. oxysporum* f. sp. *apii* on pH-adjusted oatmeal agar (15 ml) in petri plates, the fungus was transferred to the center of the agar and the colony diameter measured at daily intervals. Increase in mycelial biomass of the pathogen was also determined by growing the fungus in pH-adjusted buffered nutrient broth; mycelia were harvested on Whatman no. 1 filter paper held in a Buchner funnel; mycelial mats were dried for 3 days at 55 C and the dry weight determined.

Chlamydospore germination of *F. oxysporum* f. sp. *apii* was evaluated 18 hr after chlamydospores were introduced into a suitable pH-adjusted sterile nutrient broth medium or in the same medium colonized previously for 0, 1, 2, or 3 days by different selected soil bacteria. Spores were considered germinated when the germ tube was as long as the diameter of the spore. For each determination, three separate counts of 100 spores were made and the average percent germination determined.

The effects of pH and the *Corynebacterium* sp. on the trophic response of germinating *Fusarium* chlamydospores toward root tips of celery seedlings was also investigated. Roots of 1-wk-old seedlings of cultivar Tall Utah 52-70 R were washed in sterile distilled water and incubated for 2 hr in either a streptomycin sulfate solution (100 ppm) or in a culture of *Corynebacterium* sp. Roots with attached plants were placed in petri plates containing 10 ml of melted pH-adjusted (pH 4.5, 5.7, or 6.9) and buffered (sodium citrate) Noble agar. The molten agar contained chlamydospores at a density of 1.33×10^3/ml. Pathogen activity around the root tip was determined by counting the number of chlamydospore germ tubes impinging on root surfaces after 18 hr at 24 ± 2 C.

Measurements of disease incidence and severity. In all greenhouse experiments, inoculum was incorporated into pH-adjusted U.C. soil mix using a small mortar mixer. Five 3-wk-old plants of the highly susceptible celery cultivar Tall Utah 52-70 R were transplanted to 10-cm^3 containers and incubated on a greenhouse bench; soil temperatures varied from 21.2 to 24.3 C. After 8 wk, plants were evaluated for disease incidence and severity. Disease incidence was determined as the percent of plants showing vascular discoloration. To assess disease severity, plant stunting was determined as percent plant height in comparison with the control plants, and vascular discoloration was rated from 0 to 5 according to the method of Hart and Endo (4).

TABLE 1. The effects of pH on plant height, vascular discoloration, and disease incidence of celery cultivar (Tall Utah 52-70 R) when infected plant parts were used as inoculum to infest steamed and pH-adjusted U.C. soil mix[y]

pH of U.C. mix	Plant height (% of control)	Vascular discoloration	Disease incidence[z] (%)
7.5	47.4 a	1.6 a	80 a
6.6	31.2 c	3.7 cd	100 c
5.9	40.6 b	2.2 ab	90 b
5.6	40.0 b	2.8 bc	100 c
5.1	27.6 d	4.5 de	100 c
4.6	26.3 d	4.9 e	100 c
3.9	20.1 d	5.0 e	100 c

[y]Infected celery crowns (400 g) from a celery field were comminuted in 1,200 ml of sterile distilled water and 150-ml amounts of this inoculum mixed with 1,834 g of U.C. soil mix. Three-week-old seedlings of Tall Utah 50-70 R were transplanted; plants were incubated on a greenhouse bench and evaluated 6 wk later. Different letters represent significant differences ($P = 0.01$) according to Duncan's multiple range test.

[z]Disease incidence based on the presence or absence of vascular discoloration in the roots of 10 plants.

TABLE 2. The effects of pH on plant height, vascular discoloration, and disease incidence of celery when a mixture of macroconidia and microconidia was used to infest pH-adjusted U.C. soil mix[y]

pH of U.C. mix	Plant height (% of control)	Vascular discoloration	Disease incidence[z] (%)
7.5	97.6 a	0.0 a	0 a
6.6	80.1 a	0.7 ab	20 a
5.9	68.8 b	3.8 c	90 b
5.6	81.3 a	4.0 c	90 b
5.1	83.5 a	1.9 b	70 b
4.6	78.9 b	4.6 c	100 b
3.9	44.2 c	4.9 c	100 b

[y]Agar from five potato-dextrose plates of *F. oxysporum* f. sp. *apii* was comminuted in 1,200 ml of sterile distilled water. A 150-ml amount of inoculum was mixed with 1,834 g of each pH-adjusted U.C. soil mix; transplants were incubated on a greenhouse bench for 6 wk. Different letters represent significant differences ($P = 0.01$) according to Duncan's multiple range test.

[z]Disease incidence based on the presence or absence of vascular discoloration in the roots of 10 plants.

RESULTS

Influence of soil pH on the incidence and severity of Fusarium yellows of celery. In pH-adjusted U.C. soil mix infested with either Fusarium yellows-affected crowns or a mixture of macroconidia and microconidia, Fusarium yellows was least severe at pH 7.5 (Tables 1 and 2). As the pH was lowered, an increase in vascular discoloration and disease incidence occurred, accompanied by a decrease in plant height. Disease incidence varied from 80 to 100% when diseased celery crowns were used as inoculum, and from 0 to 100% when conidial inoculum was used (Tables 1 and 2). As the soil pH of the noninoculated control decreased from 7.5 to 3.9, plant height decreased.

When chlamydospores were used to infest U.C. soil mix, the pH of which was adjusted with buffered Hoagland's solution,

Fusarium yellows was least severe at pH 8.0 and 8.3 (Table 3). As the pH was lowered, an increase in vascular discoloration, disease incidence, and plant stunting occurred.

Influence of pH on the pathogen in pure culture. At pH 2.0 hyphae of *F. oxysporum* f. sp. *apii* failed to grow, but at pH 3.4 considerable radial growth occurred. Maximum radial growth occurred between pH 4.6 and 7.0 (Fig. 1).

Maximum mycelial growth of *F. oxysporum* f. sp. *apii* in nutrient broth after 7 days occurred at pH 5.1, but no significant difference in dry weight of mycelial mats was obtained from pH 4.0 to 9.6 (Fig. 2). Similar results were obtained in nine extensive trials when dry weights of mycelial mats were determined at 1, 2, 3, 5, and 7 days (Fig. 3A).

Influence of pH on the pathogen in dual culture with select bacteria. When *F. oxysporum* f. sp. *apii* was grown with *Corynebacterium* sp. for 7 days, maximum growth of *F. oxysporum* f. sp. *apii* occurred at pH 4.0 (Fig. 2). At a pH of 5.1 or higher, dry weight of 1-wk-old mycelial mats was significantly less. The decrease in growth was especially marked between pH 6.2 and 9.6. Similar results were obtained when *F. oxysporum* f. sp. *apii* was grown together with *Corynebacterium* sp. and dry weights of mycelial mats were determined after 1, 2, 3, 5, and 7 days (Fig. 3B). At pH 6.2 and 7.3, the weight of mycelial mats of the pathogen grown in dual cultures was greatly reduced as compared to that of *Fusarium* grown alone.

F. oxysporum f. sp. *apii* grown in dual culture with each of 24 different isolates of soil bacteria grew less at pH 7.3 than at pH 6.2 or 5.1 (Table 4). At pH 5.1, four different bacterial isolates significantly reduced mycelial growth in dual culture, whereas at pH 6.2 and 7.3, mycelial growth in dual culture was significantly reduced by 13 and 18 bacterial isolates, respectively.

Significant inhibition in chlamydospore germination occurred when chlamydospores of *F. oxysporum* f. sp. *apii* were germinated in buffered and pH-adjusted nutrient broth that had been inoculated with *Corynebacterium* sp. (Fig. 4A and B). Germination at pH 4.0 was not inhibited, but as the pH was increased to 5.1, 6.2, and 7.3, the percent of chlamydospores germinating at each successively higher pH was significantly affected; at pH 7.3 germination of chlamydospores was negligible.

Influence of streptomycin on pathogen activity near celery roots at high soil pH. The number of chlamydospore germ tubes impinging on streptomycin-treated celery roots increased significantly as the pH of Noble agar substrate was increased from 4.5 to 5.7 and 6.9 (Table 5). Conversely, there was a significant decrease in the number of chlamydospore germ tubes impinging on *Corynebacterium*-treated celery roots as the pH of Noble agar was increased over the same range (Table 5). Germination of chlamydospores in Noble agar did not differ as the pH was adjusted to 4.5, 5.7, and 6.9 and ranged from 76 to 80%. At both pH 5.7 and 6.9, there was a significantly greater increased number of hyphae impinging on root tips treated in 100 ppm streptomycin sulfate as compared with roots treated with *Corynebacterium* sp.

TABLE 3. The effects of soil pH on Fusarium yellows of celery following infestation of soil with chlamydospores of *Fusarium oxysporum* f. sp. *apii*[x]

pH of U.C. mix	Plant height (% of control)	Vascular discoloration[y]	Disease incidence[z] (%)
8.3	94.7 a	1.8 a	75 b
8.0	94.3 a	1.9 a	55 a
7.0	82.6 b	3.0 ab	95 c
6.7	78.2 b	3.7 bc	100 c
5.9	79.4 b	4.3 c	100 c

[x]Chlamydospores were incorporated into steamed and pH-adjusted U.C. soil mix at a density of 2.5×10^3 g. Three-week-old seedlings of Tall Utah 52-70 R were transplanted immediately after infesting the soil. The plants were incubated on a greenhouse bench and evaluated 6 wk later.

[y]The extent of vascular discoloration was rated from 0 to 5 according to the method of Hart and Endo (4). Different letters represent significant differences ($P = 0.01$) according to Duncan's multiple range test.

[z]Disease incidence was based on the presence or absence of vascular discoloration in the roots of 20 plants.

DISCUSSION

In experiments utilizing steamed U.C. soil mix, we demonstrated for the first time that soil pH also influenced the amount and severity of infection of celery caused by *F. oxysporum* f. sp. *apii*. This effect has been well established since numerous researchers

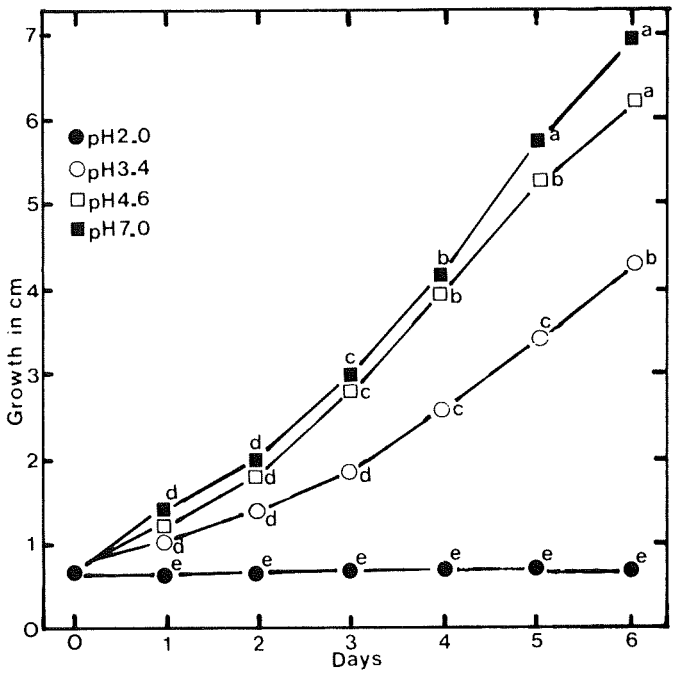

Fig. 1. The radial growth of *Fusarium oxysporum* f. sp. *apii* at various pH-adjusted levels on oatmeal agar. Different letters represent significant differences ($P = 0.01$) in daily values when compared by Duncan's multiple range test.

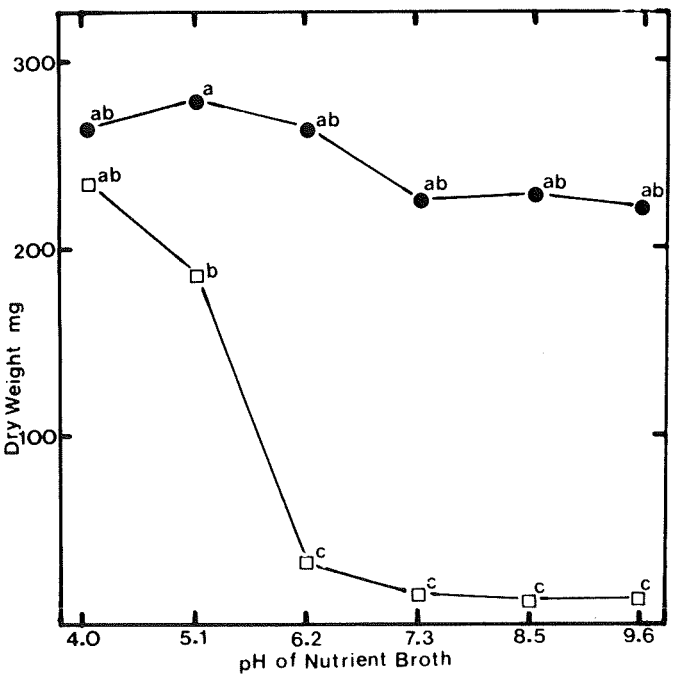

Fig. 2. The effect of pH on dry weight of mycelial mats after growth for 1 wk in nutrient broth when *F. oxysporum* f. sp. *apii* was grown alone and in dual culture with a *Corynebacterium* sp. isolated from steamed U.C. soil mix. Different letters represent significant differences ($P = 0.01$) when compared by Duncan's multiple range test.

(6–9,15,18,20,23) reported this relationship for *Fusarium*-incited diseases of banana, beans, cotton, cucumber, watermelon, tomato, and wheat.

The influence of soil pH on the incidence and severity of Fusarium yellows of celery was demonstrated to be most effective when inoculum consisted of both macroconidia and microconidia and least effective when either chlamydospores or infected celery tissues were employed as inocula. The reasons for this difference are not known, but Opgenorth (13) demonstrated that chlamydospores are much more infectious than mixtures of macroconidia and microconidia. Another possibility is that the technique employed may have failed to maintain the desired pH. However, weekly monitoring of the soil pH revealed that the soil amendment technique maintained the desired soil pH for 2 wk, whereas the irrigation technique maintained the desired pH for the entire experiment. However, the influence of soil pH appears to have been exerted during the first 2 wk of the experiment, since similar results were obtained when either chlamydospores (pH maintained by the adjusted Hoagland's solution technique) or infected celery crowns (pH maintained by the soil amendment technique) were employed as inoculum. Another possibility is that different batches of steamed U.C. soil mix used in the experiments contained variable numbers and kinds of microorganisms. This would be important because evidence is presented in this article that some, but not all, of the bacteria present in the U.C. soil mix inhibited growth and chlamydospore germination of the pathogen in in vitro tests.

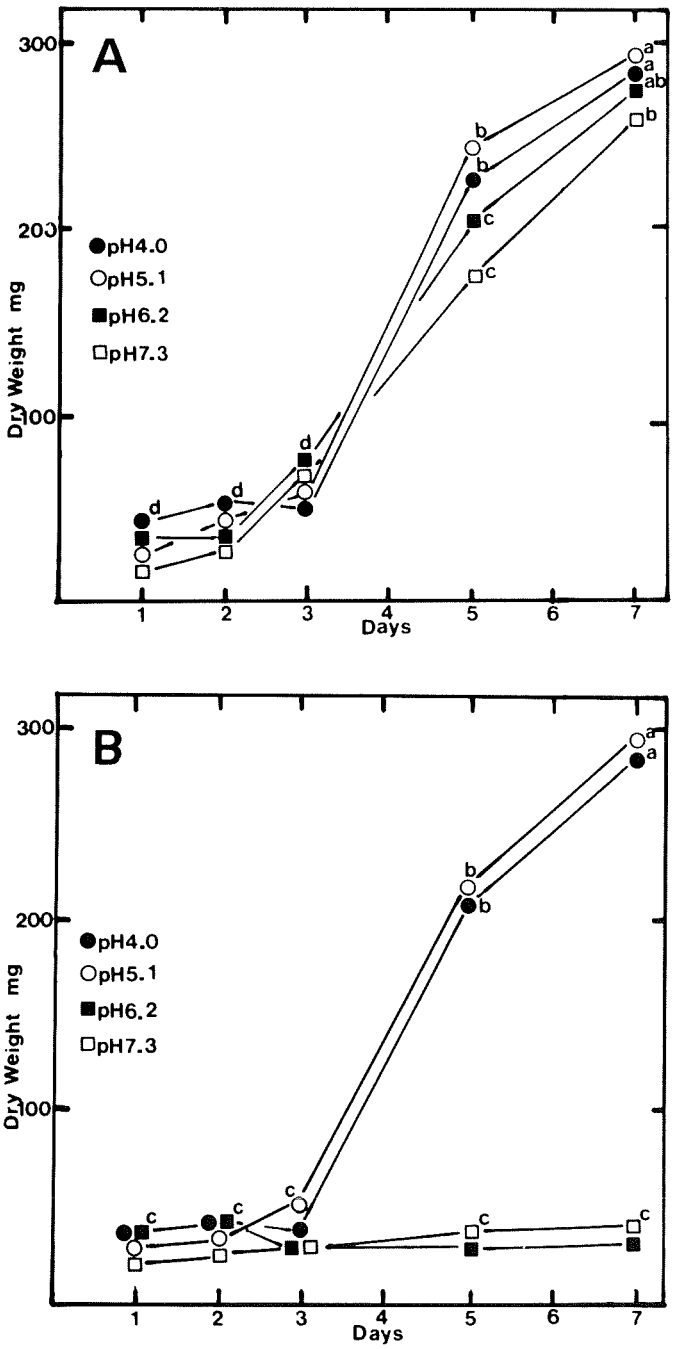

Fig. 3. The effect of pH on dry weight of mycelial mats over a 1-wk period when *F. oxysporum* f. sp. *apii* was grown in nutrient broth alone **(A)** and in dual culture **(B)** with a *Corynebacterium* sp. isolated from steamed U.C. soil mix. Different letters represent significant differences ($P = 0.05$) when compared by Duncan's multiple range test.

The lower incidence and reduced severity of Fusarium yellows of celery in slightly alkaline or neutral soil compared with acid soils apparently results from an indirect and not a direct effect of pH on the pathogen. It was determined that pH 7.0 was optimum for germination of macroconidia, microconidia, or chlamydospores and that germination decreased with increasing acidity or alkalinity. Thus, a decrease in germination of *Fusarium* propagules with increasing acidity is not consistent with an increase in disease incidence and severity at low soil pH. Similarly, a small decrease in the percent germination of propagules (ie, 5–10%) at slightly alkaline pH (7.5) cannot explain the highly significant decrease in incidence and severity of Fusarium yellows in two of three experiments (Tables 1–3) at high soil pH. Also, the high disease incidence at low soil pH and low disease incidence at high soil pH could not be explained by a direct effect of hydrogen-ion concentration on mycelial growth, because no significant differences in mycelial growth occurred when the pH of the media was varied from 4.0 to 9.6.

Because Park (14) and Marshall and Alexander (10) demonstrated competition in soil between species of *Fusarium* and various species of soil bacteria, this possibility was investigated to

TABLE 4. The effect of soil bacteria isolated from four different soils upon the mycelial growth of *Fusarium oxysporum* f. sp. *apii* when each bacterial isolate was grown in dual cultures with the *Fusarium* fungus in buffered and pH-adjusted nutrient broth

Soil type	Bacterial isolate	Dry weight (mg) after 1 wk in pH-adjusted nutrient broth		
		pH 5.1	pH 6.2	pH 7.3
	none	288	273	213
U.C. soil mix	UC 1	324	197[a]	34[a]
	UC 2	319	69[a]	33[a]
	UC 3	311	289	239
	UC 4	323	193[a]	71[a]
	UC 5	337	305	212
	UC 6	339	311	268
Chino silty clay loam	CA 1	144[a]	111[a]	85[a]
	CA 2	306	281	124[a]
	CA 3	309	289	292
	C 1	185[a]	99[a]	69[a]
	C 2	175[a]	93[a]	59[a]
	C 3	235[a]	143[a]	106[a]
Pacheco silty clay loam	PA 1	288	252	140[a]
	PA 2	317	179[a]	124[a]
	PA 3	309	266	237
	P 1	307	218[a]	65[a]
	P 2	303	242	159[a]
	P 3	304	48[a]	24[a]
Hueneme sandy loam	H 1	250	23[a]	6[a]
	H 2	301	228	158[a]
	H 3	276	230	172[a]
	H 4	273	142[a]	104[a]
	H 5	270	109[a]	91[a]
	H 6	255	235	194

[a] Designates dry weight that is significantly less than the control ($P = 0.01$) when compared with a least significant difference value of 49.05 mg.

TABLE 5. The number of chlamydospore germ tubes of *F. oxysporum* f. sp. *apii* embedded in citrate-buffered Noble agar impinging on root tips of Tall Utah 52-70 R celery seedlings treated with either 100 ppm streptomycin sulfate or an active culture of *Corynebacterium*[y]

pH of 0.1*M* citrate-buffered Noble agar	*Corynebacterium* treated[y]	Streptomycin treated[z]
4.5	5.9 bc	8.4 bc
5.7	3.5 c	11.5 ab
6.9	1.9 c	17.2 a

[y] The chlamydospore density in citrate-buffered and pH-adjusted Noble agar was 1.3×10^3 per milliliter. Different letters represent significant differences ($P = 0.01$) when compared by Duncan's multiple range test.

[z] Each value represents the mean of 10 counts made on different plants after 18 hr of incubation.

explain the effects of soil pH on the severity of Fusarium yellows of celery. Mycelial growth was significantly reduced above pH 5.1 (Figs. 2 and 3) when a *Corynebacterium* sp. isolated from steamed U.C. soil mix was transferred with the Fusarium yellows fungus to pH-adjusted nutrient broth. The same effect occurred with each of 24 different bacteria isolated from three field soils and steamed U.C. soil mix when paired with the Fusarium yellows fungus in pH-adjusted nutrient broth (Table 4). The number of bacterial isolates that significantly reduced mycelial growth increased from 16% at pH 5.1 to 54% at pH 6.2 and 75% at pH 7.3. In addition, germination of chlamydospores in pH-adjusted nutrient broth was inhibited at pH 5.1 and above when the *Corynebacterium* sp. isolated from steamed U.C. soil mix was added to nutrient broth and incubated for 2 days (Fig. 4B); at pH 7.3 germination of the chlamydospores of the pathogen was negligible. Inhibition of germination was not due to nutrient depletion since addition of glucose or asparagine failed to increase germination. Furthermore, activity of *F. oxysporum* f. sp. *apii* around root tips of celery seedlings decreased significantly when roots were treated with a suspension of the *Corynebacterium* sp. as compared with roots dipped in streptomycin sulfate at pH 6.7 and 6.9 (Table 5). Since activity of soil bacteria diminished at pH 5.5 and was almost nonexistent at pH 4.5, an increase in bacterial activity as pH rose correlated with a decrease in activity of *F. oxysporum* f. sp. *apii* as manifested by reduced mycelial growth, reduction in chlamydospore germination, and reduction of chlamydospore germ tubes associated with root tips of celery. Since these effects would be exerted on the pathogen before penetration, the effects would be manifested as a reduction in the number of infections, disease incidence, and disease severity. Hart and Endo (4) showed that severity of Fusarium yellows as evaluated by the amount of stunting and the extent of vascular discoloration was correlated with an increase in the number of infection loci.

Jones and Woltz (7,9) attributed the effect of soil pH in reducing the incidence and severity of Fusarium wilt of tomato in soils at or above pH 8.0 to unavailability of micronutrients for growth and pathogenicity of *F. oxysporum* f. sp. *lycopersici*. It was thought that the Fusarium wilt fungus caused a high incidence of infection and severe symptoms in acid soil because micronutrients were available for growth, sporulation, and infection. Although we did not design experiments to evaluate the micronutrient availability hypothesis, in two of the three pH experiments conducted with pH-adjusted U.C. soil mix, the celery plants were watered daily with a dilute nutrient solution containing all of the microelements and macroelements that Jones and Woltz (7,9) stated are necessary for the growth, sporulation, and infectivity of *F. oxysporum* f. sp. *lycopersici*. In both experiments, highly significant reductions in disease incidence and severity occurred at soil pH values of pH 7.5 (Tables 1 and 2). This suggests that micronutrient availability to the pathogen cannot account for the effects of soil pH on the severity of Fusarium yellows disease of celery. However, this alone is not sufficient evidence to refute their claims.

Our results support other observations of a suppressive effect between *Fusarium* spp. and various species of soil bacteria (11,15).

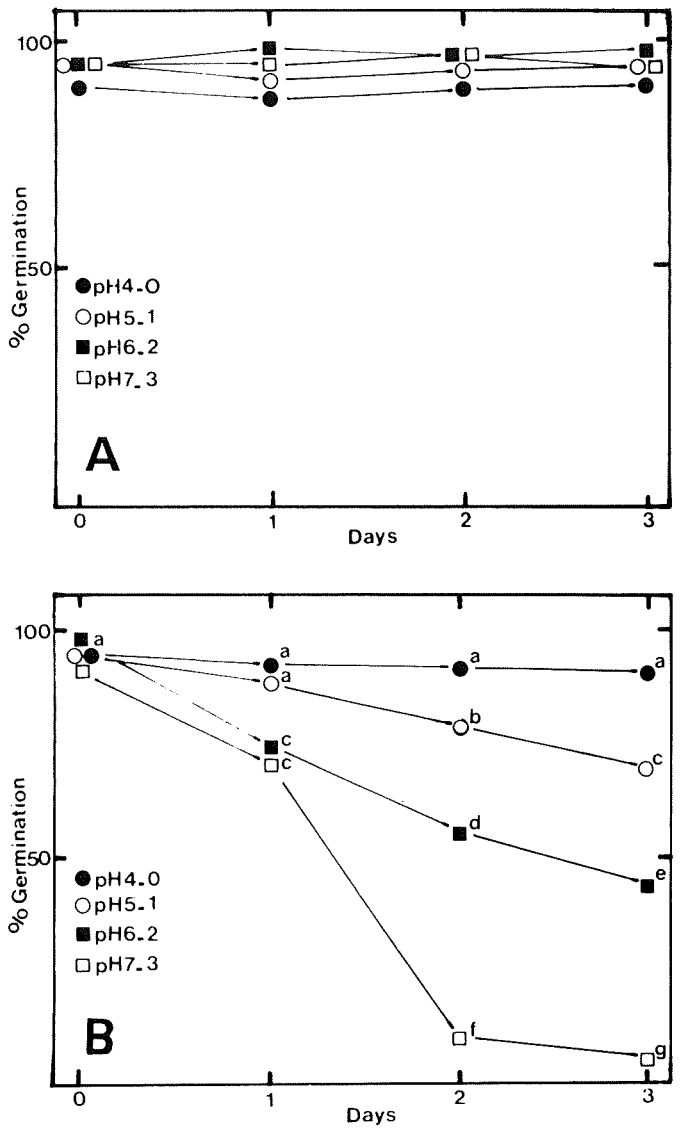

Fig. 4. Germination of chlamydospores of *F. oxysporum* f. sp. *apii* in pH-adjusted nutrient broth when the medium was initially sterile **(A)** or previously inoculated with a *Corynebacterium* sp. isolated from steamed U.C. soil mix **(B)**. Different letters represent significant differences ($P = 0.01$) when compared by Duncan's multiple range test.

That such competition is favored by soil environment, especially high pH, is also in agreement with previous work (6–8). The recent paper by Scher and Baker (16) is interesting because they presented evidence that the mechanism of control of a Fusarium-suppressive soil is probably bacterial in nature and that the relationship between soil pH and disease incidence varied depending upon the type of soil. Further work should be done in the greenhouse and field to determine if pH adjustment of soil is a practical means of controlling Fusarium yellows of celery.

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