The Role of Competition for Iron and Carbon in Suppression of Chlamydospore Germination of *Fusarium* spp. by *Pseudomonas* spp.

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Published with the approval of the director of the Colorado State University Experiment Station as Scientific Journal Series Paper 2965.
Research supported by funds from the Science and Education Administration Project 83-CRCR-1-1259, Western Regional Project W-147, and BARD Research Project US-290-80.
Accepted for publication 10 April 1985.

ABSTRACT

Elad, Y., and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75:1053-1059.

Inhibition of germination of chlamydospores of various formae speciales of *Fusarium oxysporum* in soil was correlated with production of siderophores by fluorescent pseudomonads and inhibition was totally or partially reversed by adding Fe to the system. Germination of some isolates of *F. oxysporum, F. solani*, and *F. graminearum* was also inhibited at low levels by a mutant of *Pseudomonas* spp. not producing siderophores and such inhibition was not reversed by added Fe, suggesting that competition for carbon by the bacterial biomass was instrumental in inhibition. Siderophore production was reduced proportionally more than cell division when fluorescent pseudomonads were grown in dilution series of synthetic medium containing low Fe. This suggested that in plants producing lower amounts of exudates in the rhizosphere, pseudomonads would produce lower amounts of siderophores. Germination of chlamydospores of *Fusarium* spp. in rhizospheres of nontreated plants growing in raw soil was inversely correlated with the amount of germination suppression in rhizospheres containing siderophore-producing pseudomonads. Thus, when there were more nutrients exuded in the rhizosphere, there was greater inhibition of germination of chlamydospores when pseudomonads were added to conducive soil due (by extrapolation) to production of greater amounts of siderophores by the pseudomonads. Inhibition of chlamydospore germination in soil by added pseudomonads was greater with one initial application of 1:4 glucose-asparagine (60–250 μg of glucose per gram of soil) than by an equivalent total application divided into six pulses each delivered at 2-hr intervals. The amount of inhibition induced by fluorescent pseudomonads in pulsed treatments was similar to that observed in rhizospheres of plants treated with these antagonists. Addition of available Fe reduced siderophore activity and reduced inhibition of chlamydospore germination. Disease suppressiveness in soils was not correlated with siderophore production in liquid culture by pseudomonads in systems employing *F. solani*; however, significant direct correlations among these parameters was observed for *F. oxysporum* formae speciales. Chlamydospores of *F. solani* f. sp. *phaseoli* had greater weights, volume and Fe content per propagule than those of *F. oxysporum* f. sp. *cucumerinum*. Thus, the larger chlamydospores of *F. solani* may not require exogenous Fe and energy for complete germination and successful infection.

Additional key words: biological control, soilborne pathogens.

Microorganisms compete for substrates in soil (4) especially in certain ecological niches, e.g., potential host infection courts of plant pathogens. The major elements essential for germination of fungal propagules in soil (for which antagonists may create deficiencies) are nitrogen, carbon, and iron (Fe) (3,5,12).

Competition has been implicated in suppressiveness of certain soils to Fusarium wilt diseases. Alabouvette et al (1) suggested that suppressiveness resulted from increased competition for carbon (energy sources) by the large total active microbial biomass in such soils. They postulated that suppressiveness of soil in France was due to saprophytic soil inhabitants, such as *F. solani* and *F. oxysporum*, that compete with the pathogenic *Fusarium* for limited nutrient supplies. In other studies, siderophores produced by fluorescent pseudomonads bound Fe, making it unavailable to formae speciales of *F. oxysporum* and so suppressed disease (12,19). Indeed, several *Pseudomonas* spp. inhibited germination of conidia of *F. oxysporum* f. sp. *lini* (19) and chlamydospores of *F. o.* f. sp. *cucumerinum* (24) by limiting Fe-availability in the rhizosphere. However, the role of competition for carbon and nitrogen sources by pseudomonads in those systems was not reported.

The objectives of the present study were to determine the role of carbon in competition between the growth of *Pseudomonas* spp. and the germination of chlamydospores of *Fusarium* and to compare the magnitude of such phenomena with competition for Fe in soil.

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MATERIALS AND METHODS

Growth of bacterial isolates and siderophore production. The strains of *Pseudomonas* used throughout this work were: *Pseudomonas* spp. strains 346, 27a, 381, 61, 609EC (24); *P. putida* strains A12 (19) and NIR and 8c (6); and a non-siderophore-producing *Pseudomonas* sp. strain A1/UV/AB-6 obtained from M. Schroth, Dept. Plant Pathology, University of California, Berkeley (13). *Enterobacter cloacae* was obtained from G. Harman (N.Y. State Agric. Exp. Stn., Cornell University, Geneva). Cultures were grown on nutrient agar (Becton-Dickinson Corp., Cockysville, MD) for short-period maintenance, whereas for long-term storage bacteria were kept on freeze-dried skim milk. Siderophore production was determined by growing the bacteria in low-iron synthetic medium (SM) containing 20.0 g of sucrose, 2.0 g of L-asparagine, 1.0 g of K_2HPO_4 and 0.5 g of $MgSO_4 \cdot 7H_2O$ (Sigma Chemical Co., St. Louis, MO) in 1 L of distilled water (pH 7.0) (19). Various dilutions of this medium were used as specified in the results. Cultures were grown at 28 C in 250-ml Erlenmeyer flasks each containing 50 ml of medium, in a rotary shaker at 60 rpm for 24 hr. The later procedures were done in acid-washed glassware. Liquid cultures were centrifuged at 2,500 *g* and supernatants were filtered through 0.4 μm polycarbonate membrane (Nucleopore, Pleasanton, CA) and were adjusted to 5.5 with 0.1 N HCl. The adjusted filtrate was added to two

spectrophotometer tubes (3 ml per tube). Ten microliters of fresh 10^{-2} M $FeCl_3$ solution was mixed in one tube, whereas the second tube served as a reference blank. Absorbance at 410 nm was measured with a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY). Bacterial isolates grown on SM agar medium were collected, rinsed twice by centrifugation, resuspended in distilled water to the desired concentrations and kept at 4 C until time of application in experiments.

Production of chlamydospore suspensions. Mycelial disks from cultures of *Fusarium* spp. were introduced into 50 ml of potato-dextrose broth (PDB) (Difco Laboratories, Detroit, MI) in 250-ml Erlenmeyer flasks. Cultures were incubated on a rotary shaker at 250 rpm at 28 C. After 5 days, cultures were sieved aseptically through 10 layers of cheesecloth and rinsed three times in sterile H_2O. Conidia were introduced into new PDB for 24 hr of incubation at 28 C. The germinated conidia were introduced into filter-sterilized soil extract and incubated at 27 C for 7 days. Chlamydospores were separated from mycelial fragments and conidia according to size by sieving through 20 and 7-μm Nitex Nylon screens (Tetko Inc., Elmsford, NY) and glass homogenized. Suspensions of chlamydospores were stored at 4 C until used in experiments.

Germination of chlamydospores of *Fusarium* spp. in soil. The following isolates of *Fusarium* spp. were obtained from the Department of Plant Pathology and Weed Science, Colorado State University: *F. oxysporum* Schlecht. emend. Snyd. & Hans., f. sp. *cucumerinum*, f. sp. *vasinfectum* (Alk.) Snyd. & Hans., f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans., f. sp. *conglutinans* (Wr.) Snyd. & Hans., f. sp. *melonis* (Leach et Curr.) Snyd. & Hans., f. sp. *lini* (Balley) Snyd. & Hans., and f. sp. *pisi* (Lindf.) Snyd. & Hans.; *F. solani* (Mart.) Appel. & Wro., f. sp. *cucurbitae* (Mart.) Sacc., f. sp. *phaseoli* (Burk.) Snyd. & Hans., f. sp. *pisi* (Jones) Snyd. & Hans.; and *F. graminearum* Schwabe (isolates I from wheat and II from carnation). Soil was infested with chlamydospores of the various fusaria by the method Sneh et al (24) with Fusarium wilt-conducive Ascalon sandy loam, pH 7.3 described previously (19). The soil contained 1.4×10^4–8.9×10^5 cfu/g soil of the added *Fusarium* spp. formae speciales.

To stimulate root exudation in the rhizosphere, samples of 5 g of chlamydospore-infested soils were placed in 16×22.5-cm plastic bags (Mobil Chemicals Co., Macedon, NY). Aliquots (0.1 ml) of bacterial suspensions, glucose and asparagine (Sigma) solution, $FeCl_3$ chelator solution, or H_2O were mixed with the soil at the beginning of the experiment for one application method. When nutrient solutions were applied in pulses, 0.05 ml was mixed into the soil six times at 2-hr intervals after the initial application of 0.1 ml. Concentrations of applied nutrients were adjusted so that the amount added at the beginning for the one-time application treatment was equal to the total amount added in pulses. Equivalent amounts of water were also added to the nonpulsed treatments. The bags were not sealed to prevent uneven accumulation of moisture. Soil was incubated for 20–24 hr and chlamydospore germination was assayed with an UV light microscope (Olympus, Tokyo, Japan) after staining with Calcofluor New M2R (American Cyanamid Co., Bound Brook, NJ) as described by Scher and Baker (20) and Sneh et al (24).

To test germination of chlamydospores in the rhizosphere, chlamydospore-infested soils were prepared as mentioned above and placed on 76×25-mm glass microscope slides. The following plants were used: cucumber (*Cucumis sativus* L. 'Straight Eight'), pinto beans (*Phaseolus vulgaris* L. 'Olathe'), tomato (*Lycopersicon esculentum* L. 'Big Boy'), wheat (*Triticum aestivum* L. 'Scout'), sugar beet (*Beta vulgaris* L. 'FC702/2'), radish (*Raphanus sativus* L. 'Scarlet Globe'), flax (*Linum usitatissumum* L. 'Taichung'), pea (*Pisum sativum* 'Laxton's Progress'), and corn (*Zea mays* L. 'SX756A1'). Seeds were surface disinfested in 2% sodium hypochlorite solution, washed in sterile distilled water, and germinated at 25 C on autoclaved moist paper towels in plastic bags. Roots of 5- to 7-day-old seedlings were placed in the treated soils on the slide, covered with a second glass slide, fixed in place with two rubber bands and incubated at 28 C on a moist paper towel in a plastic bag. Soil adjacent to the roots was removed after 36 hr and assayed for chlamydospore germination (19,24).

Counting bacterial population densities. Counts of bacteria added to soil or in liquid medium were assayed by plating the appropriate dilutions, by use of the drop-plate method (17), on SM agar. Counts of total population of the resident pseudomonads in nontreated soil were 1.2×10^6 cfu/g soil and this was subtracted from counts of the treated soils. Results were expressed as colony-forming units per gram of dry soil (cfu/g).

Iron content of chlamydospores. Suspensions of chlamydospores *F. o.* f. sp. *cucumerinum*, and *F. s.* f. sp. *phaseoli* were freeze-dried. Dried samples of both fungi, which contained 7.4×10^8 chlamydospores and weighed 15 and 29 mg, respectively, were acid digested in 1 ml 16 N HNO_3. After incubation at 70 C for 16 hr, 0.5 ml of the solution was diluted with 4.5 ml H_2O. Inductively coupled plasma spectrophotometry (8) was used for analysis of iron content.

Application of iron chelators. Ethylenediaminedi-*o*-hydroxyphenylacetic acid (EDDHA) was dissolved in 0.1 N KOH and adjusted to pH 6 with 0.05 N HCl. This EDDHA solution was mixed with chlamydospore suspensions or chlamydospore-enriched soil to the desired concentration. In these experiments, chlamydospores were induced to germinate by addition of glucose and asparagine (24).

Fusarium wilt suppression by bacteria. Oat-soil inocula of *F. o.* f. sp. *cucumerinum*, f. sp. *conglutinans*, and f. sp. *pisi* and *F. s.* f. sp. *phaseoli* and f. sp. *pisi* were prepared according to Scher and Baker (18). The number of propagules of *Fusarium* in the inocula was 5×10^4–4×10^5 cfu/g of soil as measured by use of Komada's medium (14). Inoculum of the *Fusarium* spp. formae speciales pathogenic to cucumber, radish, pea, beans, and pea, respectively, was mixed with air-dried conducive soil (6,19) so that inoculum densities were 250, 75, 1,000, 200, and 450 cfu/g, respectively. Bacterial isolates were applied either to pea seeds or to infested soil in which cucumber, radish, or beans were planted. Six cucumber seeds, 15 radish seeds, six pea seeds, or six bean seeds were planted in each of six to eight replicates in plastic pots (11 cm in diameter and 7 cm high) containing 250-g portions of soil infested with the appropriate formae speciales. Pots were placed on benches under continuous illumination (500 lx) at 25 ± 2 C. Plants showing disease symptoms were recorded and removed every few days. Surface-disinfested plants were planted on Komada's medium (14) to ensure correct diagnosis.

Experiments were repeated at least two times with at least four replicates. When results were the same among experiments, statistical analyses were applied to the last experiment. Experimental data were subjected to regression analysis or analysis of variance (with mean separations accomplished by Fisher's least significant difference test) as appropriate ($P = 0.05$).

RESULTS

Production of siderophores by *Pseudomonas* spp. in liquid culture. SM was made up in a tenfold dilution series. *Pseudomonas* sp. (strain 346) and *P. putida* were introduced at 1.4×10^8 and 5.3×10^8 cfu/ml, respectively (Fig. 1). After 16 hr of incubation, the population densities of the respective isolates at two- to fourfold dilutions of the basal medium were 1.4–1.3×10^9 and 2.3–2.2×10^9 cfu/ml. However, siderophore production by these isolates at these same dilutions was reduced from 0.7 to 0.18 and from 0.24 to 0.05 optical density units, respectively.

Influence of bacterial biomass in soil on germination of *F.o.* f. sp. *cucumerinum*. Five bacterial isolates with different abilities to inhibit germination of chlamydospores of *F.o.* f. sp. *cucumerinum* were added to conducive soil to which glucose-asparagine mixtures were introduced (to induce chlamydospore germination) and their growth rates determined. The bacteria were: *P. putida* strain A12, *Pseudomonas* sp. strain 346, a low siderophore-producing isolate of *Pseudomonas* sp. strain 27a, a nonsiderophore-producing mutant of *Pseudomonas* sp. strain A1/UV/AB-6, and *E. cloacae* (no siderophore production detected). Population densities (Fig. 2) at the end of the log phase (7–10 hr) for all bacterial isolates were

not significantly different ($1.0–2.0 \times 10^8$ cfu/g soil). Germination of chlamydospores in soil after 24 hr incubation compared with germination in soil to which bacteria were not added was significantly reduced by 56, 62, 31, 23, and 26%, respectively.

Effect of pulse application of glucose and asparagine into soil on the reduction of germination of chlamydospores by *Pseudomonas* sp. (strain 346). Glucose and asparagine were added to soil in concentration ratios ranging from 30:7.5 to 500:125 μg/g soil. When the nutrients were added at one initial application at the beginning of the experiment, optimum inhibition of chlamydospore germination was observed at concentration 125:41 μg/g soil (Fig. 3). When the same (total) application was divided into six pulses at 2-hr intervals, an increase in inhibition of chlamydospore germination from 13 to 37% was obtained.

Effect of Fe on growth on *Pseudomonas* sp. strain 346 in soil. The influence of pulsed application of glucose:asparagine in total concentration of 125:41 μg/g on growth of the bacterium and its effect on chlamydospore germination was examined in soil where Fe^{3+} was applied either in pulses or once at total rate of 10^{-4} mol/g of soil. Chlamydospore germination in soil not treated with *Pseudomonas* sp. ranged from 23.8–52.0% depending on the application method of the nutrients and Fe (Table 1). Moreover, chlamydospore germination when *Pseudomonas* strain 346 was added was inhibited by 21% when glucose, asparagine, and Fe were applied once at the beginning of the experiment compared with 7% when the compounds were applied in pulses as compared with controls in which pseudomonads were not applied. Inhibition of chlamydospore germination was 34 and 65% in the absence of Fe when glucose and asparagine were pulsed or applied once, respectively.

The lag phase of growth of *Pseudomonas* sp. was significantly longer in soil during the first 4 hr of incubation when Fe was applied one time or when glucose and asparagine were pulsed in the absence of Fe than in other treatments (Fig. 4).

Chlamydospore germination in rhizosphere soil of various crops. Soils containing chlamydospores of *F.o.* f. sp. *vasinfectum*, f. sp. *dianthi*, f. sp. *conglutinans*, f. sp. *melonis*, f. sp. *lini*, f. sp. *cucumerinum*, and f. sp. *pisi*; *F.s.* f. sp. *pisi*, f. sp. *cucurbitae*, and f. sp. *phaseoli*; and *F. graminearum* I and II were incubated with roots of each of the following plants in all combinations: cucumber, corn, tomato, radish, flax, sugar beet, bean, wheat, and pea in the presence or absence of isolate 346. Average inhibition of chlamydospore germination by *Pseudomonas* sp. strain 346 was 58.2–67.1% for formae speciales of *F. oxysporum*, whereas for the tested *F. solani* formae speciales it ranged from 29.5 to 37.1 and for *F. graminearum* it was 41.9–49.1% (Table 2).

Percentage of chlamydospore germination in nontreated rhizospheres was inversely correlated with suppression of germination in the presence of *Pseudomonas* sp. strain 346 ($P = 0.05$) for all the tested *Fusarium* spp. except for *F. s.* f. sp. *cucurbitae* (Table 2).

Chlamydospore germination in the rhizospheres of various plants treated with *Pseudomonas* spp. and Fe. Chlamydospores of various formae speciales of *Fusarium* spp. were placed in the rhizospheres of their respective hosts. Conducive soil was infested with the siderophore-producing strain 346 or strain A1/UV/AB-6 (that did not produce siderophores) with or without the addition of Fe. Chlamydospore germination in the presence of the mutant was reduced up to 32% in some cases and the addition of Fe had no influence on inhibition (Table 3). Strain 346, however, inhibited germination of formae speciales of *F. oxysporum* by 39.9–50.3%. The addition of Fe significantly reduced inhibition 9–31.5%. Inhibition of *F. solani* and *F. graminearum* chlamydospore germination by strain 346 was 8.1–29.8% and added Fe had no significant effect on germination.

Influence of EDDHA on germination of chlamydospores of *Fusarium* spp. EDDHA added to soil reduced germination of chlamydospores of *F. solani* and *F. graminearum* formae speciales from 5–28%, whereas those of six formae speciales of *F. oxysporum* were reduced from 36 to 70% (Table 4).

Similar inhibition was obtained with chlamydospores of these fusaria exposed to a solution of glucose:asparagine (125:41 μg/ml) and EDDHA (1×10^{-4} mol/ml) in vitro.

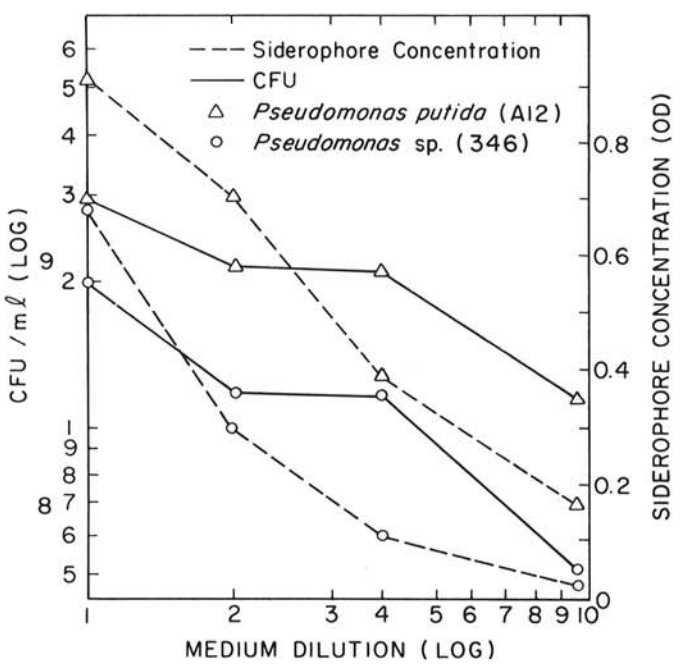

Fig. 1. Siderophore concentration (-----) and growth (——) of *Pseudomonas* sp. strain 346 (o) and *P. putida* strain A12 (Δ) during 16 hr of incubation in a 10-fold dilution series of synthetic medium.

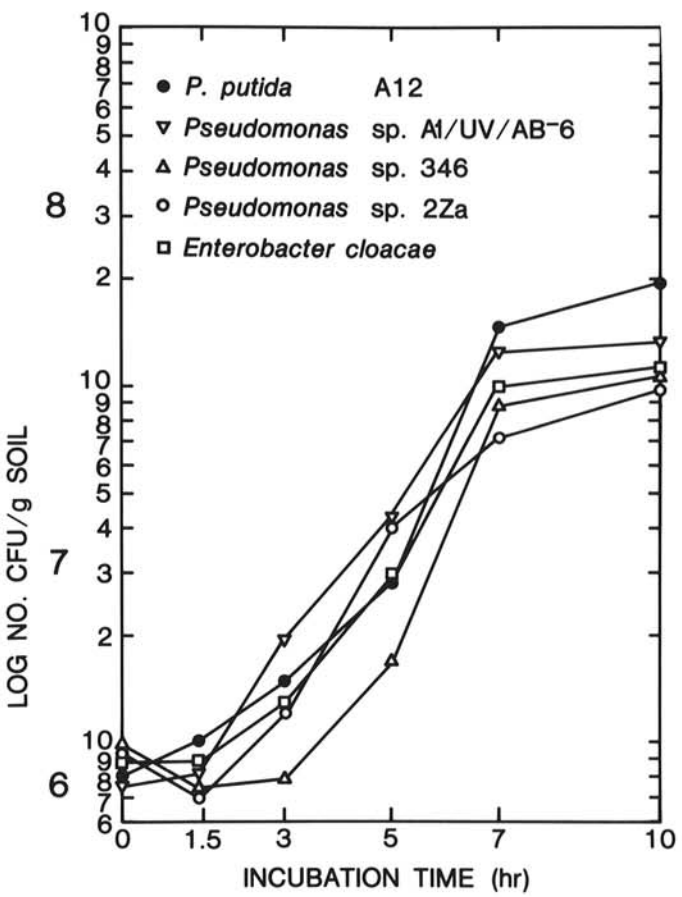

Fig. 2. Growth curves, in chlamydospore-enriched soil, of *Pseudomonas* spp. strains 346 (Δ), 2Za (o), A1/UV/AB-6 (Δ), *P. putida* A12 (•) and *Enterobacter cloacae* (□). Germination of chlamydospores after 24 hr of incubation compared with nontreated controls was reduced by 62, 31, 23, 56, or 26%, respectively (Fisher's least significant difference = 8.8%).

Iron content of chlamydospores. Chlamydospore volume, weight, and Fe content were compared for *F.o.* f. sp. *cucumerinum* and *F.s.* f. sp. *phaseoli*. The latter pathogen had an average volume of 180.05 μm^3, weighed 9.35 ng and had 0.18 ng Fe per chlamydospore. The cucumber pathogen had an average volume of 36.04 μm^3, weighed 2.03 ng, and had 0.06 ng Fe per chlamydospore.

Correlation of soil suppressiveness and siderophore production by various strains of *Pseudomonas* spp. *Pseudomonas* spp. strains A12, 346, 712, 8c, 381, NIR, 61, 609 EC, and A1/UV/AB-6 produced different amounts of fluorescent siderophore in SM. These isolates also were tested for their ability to control Fusarium wilts caused by *F.o.* f. sp. *cucumerinum*, *F.o.* f. sp. *conglutinans*, and *F.o.* f. sp. *pisi*, under environmentally controlled conditions (18). There were significant direct correlations between the amount of siderophore produced by each of the pseudomonads in culture and control of disease in cucumber ($r = 0.747$) and radishes ($r = 0.825$) when cells of the strains were mixed in soil or applied to pea seed ($r = 0.872$, Fig. 5a,b). No correlation was obtained between production of siderophore by various pseudomonads and their ability to reduce disease incidence induced by *F.s.* f. sp. *phaseoli* in beans although some isolates reduced disease incidence by 7–39%.

TABLE I. Influence of *Pseudomonas* sp. strain 346 on germination of chlamydospores of *Fusarium oxysporum* f sp. *cucumerinum* in soil when glucose and asparagine or Fe were applied in equal (total) amounts at the beginning of the experiments or in pulses[a]

Applications of Fe	Application of glucose and asparagine	Germination of chlamydospores[b]		
		Control (%)	*Pseudomonas* sp. 346[c] (%)	Inhibition (%)
None	One	49.3 w[d]	17.5 z	65
None	Six pulses	39.3 x	26.0 z	34
One	One	52.0 w	41.2 wx	21
Six pulses	Six pulses	23.8 z	22.2 z	7

[a] Glucose and asparagine or Fe were applied either once or applications were divided equally in six applications in pulses at 2-hr intervals. Total application of glucose:asparagine was 125:41 $\mu g/g$ of soil. Total application of Fe^{3+} was 1×10^{-4} mol/g soil.

[b] Germination of chlamydospores was observed after a 24-hr incubation period at 27 C.

[c] Population density dynamics of strain 346 during the course of the experiment are presented in Fig. 4.

[d] Numbers followed by the same letter are not significantly different ($P = 0.05$). There were four replications and 200 chlamydospores were counted in each replication.

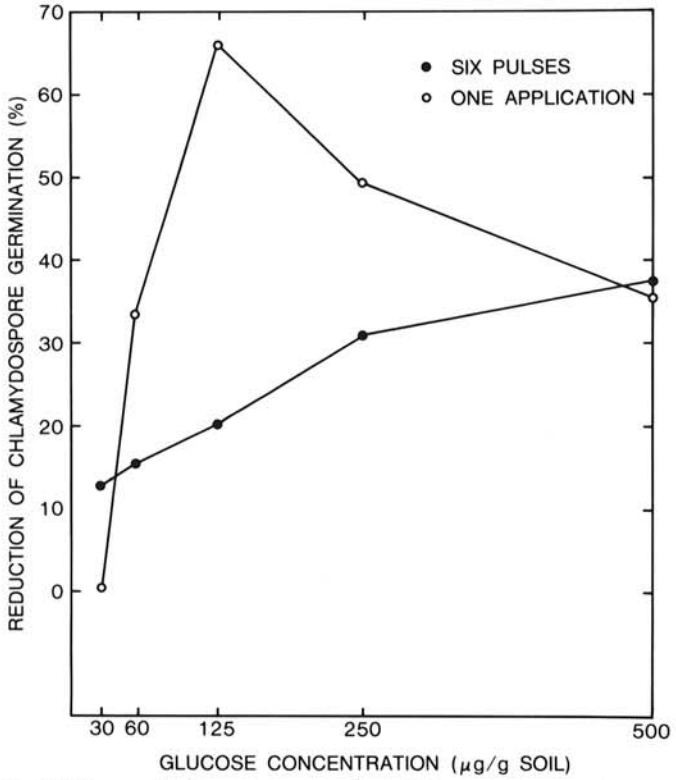

Fig. 3. Influence of different concentrations of glucose and asparagine (1:4 of each glucose concentration) on percent reduction in germination of chlamydospores of *Fusarium oxysporum* f. sp. *cucumerinum* by *Pseudomonas* sp. strain 346. Glucose and asparagine were applied once at the beginning of the 24-hr incubation (o) or in six pulses (•) (2 hr between pulses) for the same total concentration as the single application.

DISCUSSION

Two hypotheses have been advanced to explain the mechanism(s) associated with Fusarium-suppressiveness in certain soils. Kloepper et al (12) suggested that fluorescent pseudomonads in such soils produced siderophores that competed for available Fe. This reduced the efficiency for host colonization by the Fusarium wilt pathogens. Subsequent evidence (18–20,24) supported this hypothesis as reviewed in the introduction.

The other hypothesis is based on observations that more nutrients were required to induce chlamydospore germination in

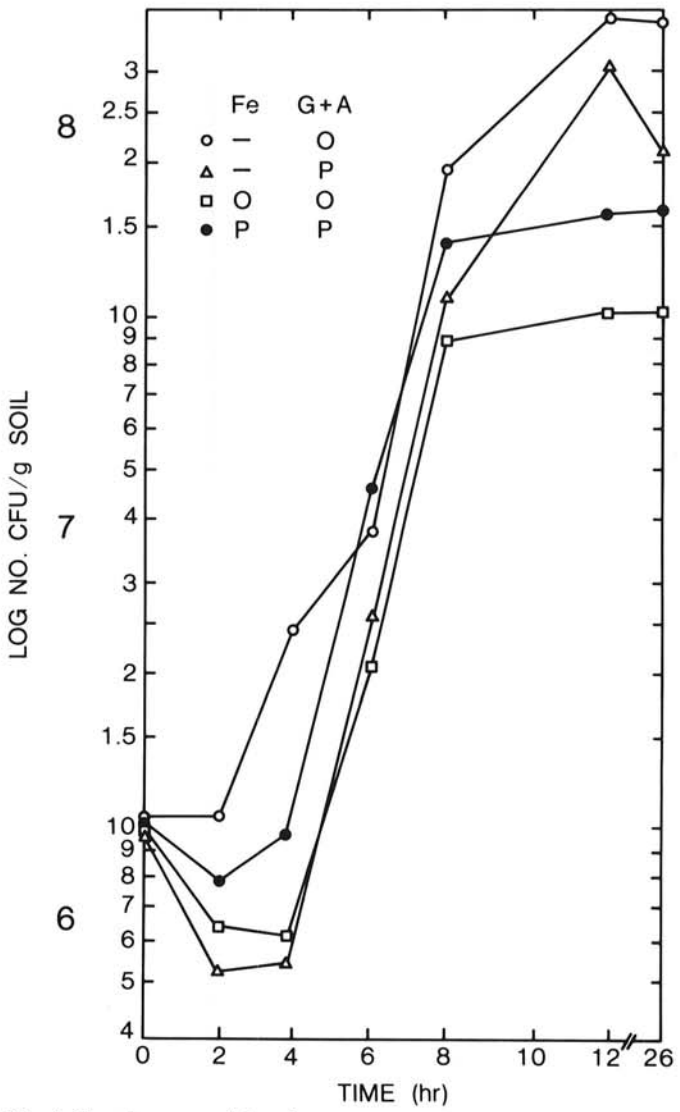

Fig. 4. Growth curves of *Pseudomonas* sp. strain 346 in chlamydospore-infested soil. Glucose:asparagine (125:41 g/g soil) were applied once (o, □), whereas Fe^{3+} (10^{-4} mol/g soil) was applied once (□), pulsed with the glucose and asparagine (•), or was not applied (o, Δ).

wilt suppressive than in wilt conducive soils (10,15,22–24). This and other observations led Alabouvette et al (1,2) to the hypothesis that the total biomass in suppressive soils reduced nutrient concentrations to the extent that the ability of chlamydospores to infect was reduced.

Growth rates of pseudomonads added in similar concentrations to soil were not significantly different (Fig. 2). Thus, the biomass contributed by the pseudomonads in each case was the same. Yet their ability to inhibit chlamydospore germination in soil (24), in the rhizosphere (Table 3), or to decrease disease (Fig. 5) differed. Such responses were directly correlated with their ability to produce siderophores. Thus, no evidence for substantial induction of pathogen or disease suppressiveness by biomass alone was obtained.

Antiobiosis in vitro (on media supplied with Fe) by any of the pseudomonads used in these investigations was not observed (24,25). However, competition for nutrient(s), other than Fe, may be involved in inhibiting chlamydospore germination since the mutant (strain A1/UV/AB-6), which did not produce siderophores, inhibited chlamydospore germination of *F.s.* f. sp. *pisi* and *cucurbitae*, and *F.o.* f. sp. *cucumerinum* in rhizospheres of host plants (Table 3). Available iron added to systems did not influence inhibition by strain A1/UV/AB-6. In contrast, where Fe was added to soil containing the siderophore-producing strain 346, inhibition of chlamydospore germination of formae speciales of *F. oxysporum* was nullified partially (Tables 1 and 3). In this case, Fe was a limiting factor—the element involved in competition. Clearly, in systems involving inhibition induced by the mutant (not producing siderophores), the limiting factor was not Fe. Rather some other nutrient essential for complete germination of chlamydospores was not available. Such observations may provide information on the impact of biomass on suppressiveness but it is obvious from a study of the data in Table 3 that such effects are quantitatively lower than the more specific mechanism of Fe-competition.

What nutrient is likely to be limiting if not Fe? Other factors essential for germination of propagules of *Fusarium* spp. in soil are carbon and nitrogen (7). Since nitrogen was in excess in the soil and in the systems involved in this research, the most likely limiting factor was carbon (5).

In the past, experimenters have applied one application of various organic nitrogen and carbon sources to induce chlamydospore germination of *Fusarium* spp. in soil (e.g., 21–23). Obviously, this does not reflect the situation found in soils where root exudates triggering germination are continuously introduced into certain areas of the rhizosphere (7). For a closer simulation of this situation, nutrients were supplied in pulses and striking differences were observed in germination inhibition (induced by addition of strain 346 to soil) between this treatment and a one-time application of nutrients at the beginning of the experiment. Except at either low or high concentrations (where no differences were observed), inhibition of germination was less when nutrients were pulsed than when applied in total at one time (Fig. 3). This provides

TABLE 2. Germination of chlamydospores of various *Fusarium* spp. in the rhizospheres[a] of plant in soil with or without the addition of *Pseudomonas* sp. (strain 346)[b]

Fusarium spp. and formae speciales	Average inhibition[b] (%)	Correlation between germination and inhibition[c] (*r*)
F. oxysporum f. sp.		
vasinfectum	59.2	0.80
dianthi	63.2	0.69
conglutinans	58.2	0.79
melonis	67.1	0.92
lini	61.9	0.81
cucumerinum	59.9	0.76
pisi	59.0	0.78
F. solani f. sp.		
pisi	32.9	0.65
cucurbitae	29.5	0.49
phaseoli	37.1	0.70
F. graminearum[d] I	49.1	0.92
F. graminearum II	41.9	0.85

[a] Roots of germinated plants were placed on chlamydospore-enriched soil between two glass slides. Germination of chlamydospores in the rhizosphere of each plant was observed after a 36-hr incubation period at 28 C.

[b] Average inhibition in rhizospheres of cucumber, corn, tomato, radish, flax, sugar beet, bean, wheat and pea. Germination among nontreated plant rhizospheres varied from 12.2–64.3%. Figures are averages over all observations for a single isolate of *Fusarium* spp.

[c] Numbers represent correlation coefficients between chlamydospore germination in nontreated rhizosphere soil and the inhibition induced by application of strain 346. All *r* values are significant ($P = 0.05$) except for *F. solani* f. sp. *cucurbitae*.

[d] Culture I of *F. graminearum* was isolated from wheat: culture II was isolated from carnations.

TABLE 3. The effect of Fe^{3+}[a] and *Pseudomonas* spp.[b] with (strain 346) and without (strain A1/UV/AB-6) the ability to produce siderophores and Fe on germination of chlamydospores of *Fusarium* spp. in rhizosphere soil[c]

		Chlamydospore germination (%)					
		Control		A1/UV/AB-6		346	
Species of *Fusarium*	Plant rhizosphere[d]	No added Fe^{3+}	Fe^{3+} added	No added Fe^{3+}	Fe^{3+} added	No added Fe^{3+}	Fe^{3+} added
F. oxysporum f. sp.							
lini	Flax	34.3	36.1	29.1	31.0	12.9*	28.4*
conglutinans	Radish	41.2	47.3	41.2	43.7	20.5*	37.5*
cucumerinum	Cucumber	46.3	43.4	31.4*	31.7*	23.9*	31.7*
pisi	Pea	42.1	51.3	37.3	41.2	25.3*	39.1*
F. solani f. sp.							
pisi	Pea	50.0	48.1	38.4*	40.0*	35.1*	34.4*
cucurbitae	Cucumber	38.1	39.4	32.3*	30.1*	34.0	30.4*
phaseoli	Bean	27.1	26.4	28.3	26.9	22.3*	23.6*
F. graminearum[e] I	Wheat	27.2	33.4	34.1	32.2	25.0	26.4
F. graminearum II	Corn	36.7	35.7	32.0	33.3	29.6	31.0

[a] Fe^{3+} concentration was 2×10^{-4} mol/g of soil.

[b] Population density of both *Pseudomonas* spp. strains initially was adjusted to 9×10^{6} cfu/g of soil.

[c] Values marked with an asterisk (*) are significantly different ($P = 0.05$) from the control.

[d] Roots of germinated plants were placed on chlamydospore-infested soil between two glass slides. Germination of chlamydospores in the rhizosphere of each plant was observed after 36-hr incubation at 28 C.

[e] Culture I of *F. graminearum* was isolated from wheat; culture II was isolated from carnation.

at least a partial explanation of the results of Sneh et al (24) who observed less inhibition induced by fluorescent pseudomonads in the rhizosphere than in tests in which soils were supplied with one application of nutrients.

The explanation for these results may be complex but certain hypotheses may be advanced. First, nutrient concentration (in vitro) had a profound effect on the production of siderophores; the less nutrient the lower the proportion of siderophores produced per unit of multiplication of a pseudomonad (Fig. 1). This is not surprising since siderophores are secondary metabolites (11,16) and require adequate carbon levels to sustain production. Thus, small pulses of nutrients applied experimentally to soil or in the rhizosphere would supply less carbon to support activity of pseudomonads during the first critical period of chlamydospore germination induction than would a one-time application of nutrients. This is reflected by results presented in Fig. 4; one application of glucose-asparagine resulted in a higher growth rate (especially 2–6 hr after application) than when there were six applications in pulses. In this same experiment (Table 1), inhibition of chlamydospore germination by strain 346 in a one-time application of nutrients was 65%; when pulsed it was 26%. This difference in inhibition originated from two variables: first, chlamydospore germination level in soil not supplied with strain 346 and amended once with glucose-asparagine was higher than when these nutrients were applied in pulses. Second, the reverse was true for soil infested with this siderophore-producing isolate. This apparent inverse correlation was also noticed in experiments involving many strains of *Fusarium* spp. germinating in rhizospheres in soil infested or not infested with the siderophore-producing strain 346 (Table 2). There was a significant direct correlation between the germination of chlamydospores in soil to which strain 346 had not been added and the inhibition induced when the strain was introduced. This suggests that the more nutrients applied (as reflected by higher levels of chlamydospore germination) the more inhibition induced by the pseudomonad. Therefore, increased inhibition results in soil when more nutrients (except Fe) are available for the production of siderophores (Fig. 1). Similarly, Howell and Stipanovic (9) found seed treatment with *Pseudomonas fluorescens* was effective as a protectant and produced pyluteorin when the cells had an available nutrient source.

Secondly, a further test of these conclusions may be performed by varying the Fe-availability in soil. Fluorescent pseudomonads produce siderophores only in low iron environments (16). Fe supplied in either pulses or at one time, partially nullified inhibition of chlamydospore germination induced by strain 346 (Table 1). Thus, even though chlamydospore germination was increased by one application of glucose-asparagine plus Fe over that in the pulsed treatment, one application of Fe depressed activity of strain 346 more than any other treatment (Fig. 4). Depression of

TABLE 4. The effect of EDDHA on germination of chlamydospores[a] of *Fusarium* spp. in soil

Species of *Fusarium*	Germination (%)[b]	
	EDDHA not applied	EDDHA applied[c]
F. oxysporum f. sp.		
vasinfectum	32.3	15.7*
conglutinans	48.2	24.3*
melonis	45.4	25.4*
lini	41.3	12.4*
cucumerinum	31.4	20.2*
pisi	47.3	27.2*
F. solani f. sp.		
pisi	38.3	31.7
cucurbitae	26.3	28.9
phaseoli	31.8	30.8
F. graminearum[d] I	26.7	28.3
F. graminearum II	44.1	38.3

[a] Chlamydospores were induced to germinate by adding 125:41 μg glucose: asparagine per gram of soil. Measurements of germination were obtained 20 hr after initiation of the experiment. Incubated at 28 C.

[b] Numbers marked with an asterisk (*) were significantly ($P = 0.05$) different from controls where EDDHA was not supplied. There were four replications and 200 chlamydospores were counted in each replication.

[c] EDDHA was applied at 3×10^{-4} mol/g soil.

[d] Culture I of *F. graminearum* was isolated from wheat; culture II was isolated from carnation.

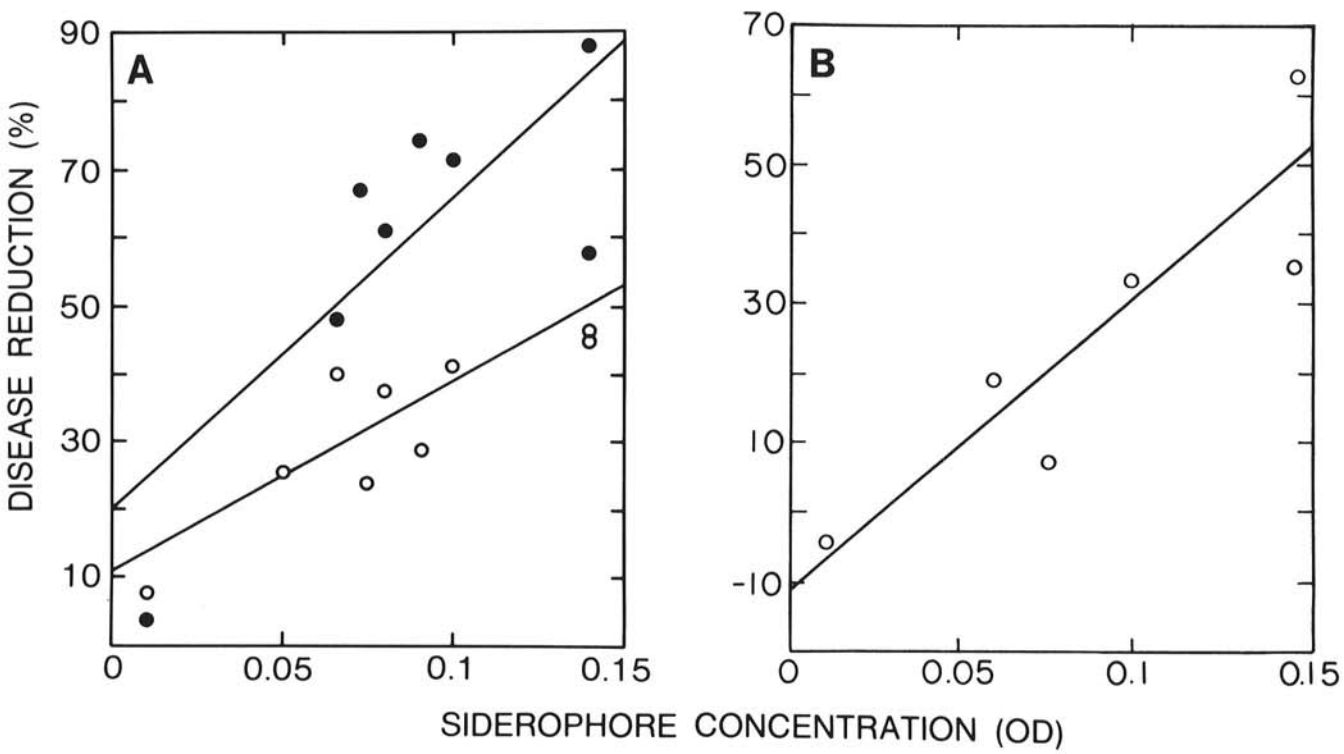

Fig. 5. Correlations between siderophore production by various isolates of *Pseudomonas* spp. and suppression of Fusarium wilts induced by: **A,** *F. oxysporum* f. sp. *cucumerinum* in cucumber ($r = 0.747$, •) or f. sp. *conglutinans* in radish ($r = 0.825$, o); **B,** *F. oxysporum* f. sp. *pisi* in peas ($r = 0.872$).

pseudomonads by Fe was also noticed in rhizospheres of radishes in monoculture when a chelated Fe compound with a low stability constant was added to the soil (21).

Suppressiveness to the formae speciales of *F. oxysporum*, but not to other species of *Fusarium* (22,23), was observed in the field. Low levels of inhibition of chlamydospore germination were noticed in this study for some isolates of *F. solani*. This was attributable to competition for carbon but not Fe. Again, addition of the ligand, EDDHA (that binds iron in soil so that it is not available to the hydroximate siderophores of *Fusarium* spp. [16,19]), suppressed germination of isolates of *F. oxysporum* but not that of *F. solani* or *F. graminearum* (Table 4).

Why did low availability of Fe affect germination of formae speciales of *F. oxysporum* but not *F. solani* and *F. graminearum*? In general, chlamydospores of *F. oxysporum* are smaller than in the other species. Indeed, when chlamydospores of *F.s.* f. sp. *phaseoli* and *F.o.* f. sp. *cucumerinum* were compared the ratios of volume were 4.99:1, weight 4.6:1, and Fe 3:1. This suggests that the larger chlamydospores of *F. solani* contained more Fe per propagule than those of *F. oxysporum* and, thus, did not require an exogenous supply of this element.

Identification of mechanisms associated with the induction of suppressiveness in soil to the Fusarium wilt and take-all pathogens has been explored extensively in this and other research articles (18–21,24,25). No evidence for participation of lysis (24) or antibiosis (25) was obtained. The only mechanism so far clearly identified that induces substantial suppressiveness in soil is competition for Fe.

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