Ecology and Epidemiology

Association of Chemical and Biological Factors in Soils Suppressive to *Pythium ultimum*

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ABSTRACT

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Suppression of saprophytic increases in inoculum densities of *Pythium ultimum* was found in finely textured soils in the San Joaquin Valley of California. Suppressive and conducive soils showed no consistent relationship between suppressiveness and soil pH, concentrations of calcium, magnesium, or potassium, cation exchange capacity, or electrical conductivity. Whereas suppressive soils had greater mean concentrations of sodium, sulfate, and chloride than conducive soils, only chloride was inhibitory to *P. ultimum*. When conducive soils were amended with chloride at concentrations found in suppressive soils, colonization of leaf debris by *P. ultimum* was partially suppressed. Suppression of debris colonization coincided with a significant increase in the frequency of colonization by *Pythium oligandrum*. *P. oligandrum* was active as a saprophyte sooner in chloride-amended soils than in field soils and was significantly more tolerant of chloride than *P. ultimum*. In suppressive soils, *P. oligandrum* was the most commonly isolated primary colonizing fungus and tended to be found at higher propagule densities than observed in conducive soils. When propagule densities of *P. oligandrum* were increased artificially in conducive soils, colonization and subsequent inoculum increases of *P. ultimum* were reduced. Suppressiveness was overcome by successive soil amendments with dried leaf debris, which resulted in progressive reductions in the frequencies of colonization by *P. oligandrum*. Apparently soils with elevated chloride concentrations allowed *P. oligandrum* to successfully compete with *P. ultimum* and, thus, increase its propagule density and further suppress the saprophytic activity of *P. ultimum*.

Soilborne organisms exist in a biologically buffered ecosystem in dynamic equilibrium among themselves as influenced by their physical environment (11). Changes in the environment can shift the equilibrium to favor one group of organisms and disfavor another. When environmental conditions favor organisms antagonistic to plant pathogens, soil suppressiveness may occur. The monoculture of radish induced suppressiveness to *Rhizoctonia solani* Kühn by allowing increases in the propagule densities of the antagonist *Trichoderma harzianum* Rifai (25). Suppressiveness also was induced by manipulation of the environment and acidifying the soil to favor the antagonist. This occurs under natural conditions as well, with the acid soils of Colombia favoring the saprophytic activity of *T. hamatum* (Bon.) Bainier and allowing its inoculum increases, thus rendering the soils suppressive to *R. solani* (9).

Bouhot et al (6,7) observed a more generalized form of soil suppressiveness based on the numbers of nonspecific microorganisms competing with the saprophytic activities of *Pythium* spp. In soils with high propagule densities of other primary colonizing fungi and, hence, greater competition, *Pythium* spp. had a low saprophytic activity and subsequently caused less disease. Alabouvette et al (1,2) have proposed a similar relation-

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ship with saprophytic forms of *Fusarium oxysporum* Schlect. and antagonistic fluorescent Pseudomonads involved with natural soil suppressiveness to *F. o.* f. sp. *melonis* Snyd. & Hans.

In the San Joaquin Valley of California, *Pythium ultimum* Trow is a major cause of preemergence damping-off of cotton and other crops (14). Inoculum densities of the pathogen fluctuate on a seasonal basis, with the largest increases often occurring in the fall after harvest with the incorporation of crop debris into the soil and the onset of seasonal rains (17). This saprophytic activity is an important part of the pathogen life cycle in renewing inocula in the soil. As primary colonizing sugar fungi (sensu Garrett) (15), *Pythium* spp. are poor saprophytes on organic matter previously colonized by other microbes (5,20). Therefore, factors suppressing rapid colonization of crop debris by this fungus reduce its saprophytic activity and increases of inocula.

Hancock (17) observed several sites cropped to cotton in the San Joaquin Valley that regularly had low inoculum densities of *P. ultimum*. These inoculum densities remained low even after incorporation of crop debris. This was in contrast to adjacent soil types where the inoculum densities of *P. ultimum* increased. The suppressiveness to saprophytic inoculum increase of *P. ultimum* was unaltered by flooding, dilution of the soil with sand, or mild heat treatment (18). However, a green manure with barley applied at monthly intervals for 3 mo converted a suppressive soil into a conducive soil. The current study was undertaken to elucidate the basis of suppressiveness in these soils and to investigate the involvement of biotic and abiotic factors in this phenomenon. A preliminary report has been published (27).

MATERIALS AND METHODS

Soil collection. Collection sites in the San Joaquin Valley of California, previously investigated by Hancock (18), were resurveyed in early fall after cotton harvest for three successive years beginning in 1980. Soil samples were collected only from those sites currently cropped to cotton, and the surveyed soils all belonged to Oxalis silty clay, Lethent silty clay loam, or Panoche clay loam soil series. Twenty subsamples from the top 50 mm (in 1981 the soil was collected from 0 to 120 mm with a 25-mm-diameter soil probe) were bulked, air-dried, mixed, and ground to pass a 1.0-mm-mesh sieve. All soils were stored in plastic bags at room temperature until used.

The suppressiveness of soils toward *P. ultimum* was determined by amending triplicate samples of 75 g of soil with 0.25 g of crushed cotton leaves (0.83–0.99 mm diameter) then incubating them in Styrofoam cups in a moist chamber at −0.3 to −1 bar and 21 C. After 7 days inoculum densities were determined by the soil drop assay of Stanghellini and Hancock (32) and expressed as germinable propagules per gram of soil. Those soils having net increases of inoculum density greater than 200 propagules per gram of soil were classified as conducive soils, those having increases from 21 to 199 propagules per gram of soil were classified as moderately suppressive soils, and those with less than 20 propagules per gram were classified as suppressive soils (18).

Soil chemical analysis. Saturation extracts and triple 1.0 N ammonium acetate (pH 7) extracts of all soils were performed (30). Determination of calcium, magnesium, sodium, and potassium concentrations were done for both extracts by using an atomic absorption spectrophotometer (Perkin-Elmer Model 372). Chloride concentrations in the saturation extracts were determined by titration with silver ions by using a Buchler chloridometer (30) and sulfate concentration by using a barium chloride-gelatin turbimetric procedure (35). Soil pH was determined by placing electrodes in saturation paste after equilibration for 4 hr (30). Cation exchange capacity (CEC) was determined by the methods of Bowers and Gschwend (8).

Effect of ions in the soil on *P. ultimum*. To determine the effects of sodium, chloride, or sulfate on the activity of *P. ultimum*, 25 g of a conducive soil was amended with 0.08 g of crushed cotton leaves (0.83–0.99 mm diameter) and placed in a 60-mm brass ring on a −0.5 bar ceramic tension plate. The tension plate was flooded with either distilled water, 0.03 M NaCl, or 0.03 M Na_2SO_4 and the matric potential adjusted to −0.1 bar in a pressure plate extractor. After 12 hr equilibration the soil was placed in one-half of a 60-mm-diameter plastic petri dish and covered with polyethylene plastic held in place with a rubber band. After 7 days incubation in a moist chamber (a plastic bag containing moist paper towels) at 21 C, the soil was air-dried and subsequent changes in the inoculum density of *P. ultimum* were determined by the soil drop assay method (32). Determinations of sodium, chloride, and sulfate concentrations were done as previously described.

Colonization of organic matter. Colonization of organic matter by soil fungi was investigated by a procedure similar to the one described above by using a pressure plate extractor to adjust the matric potential. At designated time intervals, triplicate samples were removed from the moist chamber and the leaf fragments recovered from the soil by wet sieving on a 0.5-mm-mesh sieve. The leaf fragments were plated on 2% water agar amended with 0.1% Tergitol NP 10 (Union Carbide Co., NY) to restrict fungal growth. After 24 and 48 hr the plates were observed and the characteristic colonies of various *Pythium* spp. counted. Those colonies that could not be positively identified by their morphology were hyphal tipped and plated on oatmeal agar-water slants for identification (17).

To study the effect of increasing chloride concentrations and different chloride salts on the patterns of leaf colonization, 0.03–1.0 M concentrations of chloride salts was used to flood tension plates before adjustment of matric potential. Final concentrations of chloride in the soil were determined as previously described. This experiment was repeated three times.

Determination of the propagule densities of *P. oligandrum*. As other media previously reported for the recovery of *Pythium* spp. from the soil proved unsatisfactory for isolation and easy identification of *P. oligandrum* Drechsler, a new medium was devised. It contained 17 g of Difco cornmeal agar, 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate, Sigma Chemicals), 100 μg/ml of penicillin G (K salt, Calbiochem, La Jolla, CA), 200 μg/ml of vancomycin HCl (Sigma Chemicals, St. Louis, MO), 50 μg/ml of rose bengal, 20 μg/ml of pimaricin (Delvocide, 50% ai, Gist-Brocades, Delft-Holland), and 20 μg/ml of benomyl (50% ai, WP, E. I. DuPont de Nemours, Wilmington, DE) in 1 L of water. The Tween 20 was added after removal of the medium from the autoclave and the antibiotics were added after the media had cooled to 40 C. All antibiotics were prepared as water solutions and stored at 4 C. After pouring, plates were stored in the dark for no more than 1 wk until used.

To test the efficiency of this medium for the recovery of *P. oligandrum*, field soil with a low propagule density (as determined by leaf debris colonization) was amended with autoclaved soil that had been reinfested with *P. oligandrum* to give known propagule densities of *P. oligandrum*. Two grams of this soil was added to 10 ml of distilled water and mixed for 15 sec with a Vortex mixer. After standing for an additional 10 min, the solution was mixed for 60 sec and 0.2 ml was plated on the surface of 3-day-old plates of the differential medium. After 48 hr incubation at 30 C, soil residue was washed from the agar surface under a gentle stream of water and colonies in the agar were counted. Final counts and observations of the plates were made after 72 hr incubation. For the determination of propagule densities of *P. oligandrum* in the field, soils were collected and handled as previously described. All soils were assayed in triplicate on a total of four plates per replicate. To test the efficiency of recovery of *P. ultimum* from field soil, the inoculum densities obtained by using the differential medium were compared with the soil drop assay. For greater sensitivity with the soil drop assay, soils with low inoculum densities *P. ultimum* were assayed by using 8 g of soil per 100 ml of H_2O, with 2 ml plated on eight water agar plates for each replicate.

Soil infestation with *P. oligandrum*. *P. oligandrum* was grown on oatmeal agar-water slants (17) for 2–3 wk before harvesting mycelium. Hyphal mats were blended for 60 sec and centrifuged at 5,000 *g* for 5 min. Pellets were resuspended in distilled water and recentrifuged. This oospore rinsing procedure was repeated three times. After the final rinse the oospores were added to autoclaved field soil, which was moistened to field capacity and air-dried at

room temperature. After being ground to pass a 0.5-mm-mesh sieve, the soil was stored at 4 C. Propagule densities of *P. oligandrum* were determined prior to use by dilution plating on the differential medium and expressed as propagules per gram of soil. The same procedure was followed in reinfesting autoclaved soil with *P. ultimum*.

Influences of *P. oligandrum* on saprophytic inoculum increases of *P. ultimum*. Propagule densities of *P. oligandrum* were increased in conducive field soil by amendment with autoclaved soil reinfested with *P. oligandrum*. Final propagule densities of *P. oligandrum* were determined as previously described. Soils were amended with crushed cotton leaves (0.83–0.99-mm diameter) at a rate of 0.08 g per 25 g of soil and the matric potential adjusted to −0.1 bar with the pressure plate extractor and incubated in a moist chamber as previously described. At set time intervals, samples were removed and the patterns of leaf colonization determined with the organic matter retrival method. After 7 days the soils were air-dried and net increases in inoculum densities of *P. ultimum* were determined.

Converting a suppressive to a conducive soil. Seventy-five grams of suppressive soil was amended with 0.25 g of crushed cotton leaves (0.83–0.99 mm diameter) and incubated between −0.1 and chamber. At days 1, 3, and 7, triplicate samples were removed and divided in half. One-half of the sample was air-dried and assayed for inoculum densities of *P. ultimum*, whereas colonization of organic matter was measured in the other half by the wet sieving method. On day 7, the remaining soils were air-dried, ground to pass a 1.0-mm-mesh sieve, reamended with crushed cotton leaves, and incubated in the moist chamber. On days 1, 3, and 7, triplicate samples were assayed as in the previous cycle. This cycle was repeated to give a total of three leaf debris soil amendments. All experiments were repeated at least twice unless otherwise noted.

RESULTS

The ratings for soil suppressiveness to *P. ultimum* of sites collected in 1981, with a few exceptions, agreed with the ratings reported in 1977 (18). The finer textured Oxalis silty clay and Lethent silty clay loam soils were predominantly suppressive soils and the coarser textured Panoche clay loam soils were predominantly conducive (Table 1). At sites where changes in ratings did occur, with one exception, they changed by only one class, e.g., soils from sites 6, 7, 22, 103, and 110 changed from moderately suppressive to suppressive, soil from site 15 changed from a conducive to moderately suppressive and soils from sites 4 and 10 changed from moderately suppressive to conducive. However, in 1977, soil from site 26 was rated as a conducive soil with a net increase of over 200 propagules per gram of soil after leaf amendment. In 1981, this soil had a net increase of eight propagules per gram of soil, classifying it as a suppressive soil.

Conducive soils were found to have a greater potential for saprophytic increases in inoculum density of *P. ultimum* than did suppressive or moderately suppressive soils. Even when nondetectable levels of the pathogen were observed in field soils before organic matter amendment (sites 3, 13, 14, 27, 30, 32, and 33), subsequent saprophytic increases in inoculum density of greater than 200 propagules per gram of soil occurred (Table 1). This was not observed with moderately suppressive or suppressive soils, even though the pathogen was present.

Soil chemical analysis. There was no association between suppressiveness and concentrations of exchangable calcium, magnesium, potassium, electrical conductivity, and soil pH (Table 2). Suppressive soils also tended to have greater cation exchange capacity than did conducive soils (84% had greater than 26 meq per 100 g vs 84% less than 22 meq per 100 g, respectively). However, this is believed to be a reflection of the difference in soil type and is not directly involved in soil suppressiveness. More significant associations were found between suppressiveness and concentrations of sodium, sulfate, and chloride, which tended to be present in greater concentrations in suppressive than in conducive soils (Tables 2 and 3). Exchangeable sodium concentrations from samples collected in 1981 ranged from 0.8 to 5.3 meq per 100 g in suppressive soils and from 0.5 to 2.7 meq per 100 g in conducive soils. Sulfate concentrations in the saturation extract ranged from 1.0 to 12.6 meq/L in suppressive soils and from 0.3 to 6.8 meq/L in conducive soils. Although suppressive soils tended to have greater concentrations of these ions than did conducive soils, there was only a slight negative correlation between saprophytic inoculum increase of *P. ultimum* and ion concentration ($r = -0.12$ and $r = -0.29$, $P = 0.05$, for sodium and sulfate, respectively).

More consistent relationships between suppressiveness and ion concentration were found with chloride (Table 3). In suppressive soils, chloride concentrations ranged from 2.6 to 55.6 meq/L for the 3 yr of sampling, with 80% of the surveyed soils having concentrations greater than 10.6 meq/L. These concentrations were greater than those found in conducive soils, where 64% were below 10.6 meq/L. Those soils collected in 1981 tended to have lower chloride concentrations than other collection dates due to the depth at which soil samples were taken. However, the mean chloride concentrations of suppressive soils were still greater than conducive soils for this collection date. Chloride concentrations found in moderately suppressive soils were in the same range as those found in conducive soils (e.g., 3.0–13.9 meq/L). For soils collected in 1981 (the year with the lowest chloride concentration), there was a slight negative correlation between chloride concentrations and saprophytic inoculum increases of *P. ultimum* ($r = -0.29$, $P = 0.05$, and slope $= -0.004$).

Effect of ions in the soil on *P. ultimum*. When conducive soils were amended with either NaH_4PO_4 or Na_2SO_4 there were no significant differences in net increases of inoculum density of *P. ultimum*, indicating that at the concentrations found in suppressive soils, these ions have no detrimental effect on the saprophytic activity of *P. ultimum* (Table 4). However, when soil was amended with chloride in the form of NaCl, there was a significant suppression in net increases of inoculum of *P. ultimum*

TABLE 1. Increases in inoculum densities of *Pythium ultimum* after amendment of field soil with cotton leaf debris[a]

Soil classification	Site	Soil type[b]	Inoculum density (p/g soil)	
			Initial	Final
Conducive	3	L	ND[c]	581
	4	L	17	346
	10	P	ND	208
	12	P	17	1,025
	13	O	ND	354
	14	P	ND	354
	27	P	ND	536
	30	P	ND	317
	31	P	58	525
	32	P	ND	528
	33	P	ND	613
Moderately suppressive	15	O	ND	72
	25	L	ND	25
	35	L	ND	29
	100	L	ND	39
Suppressive	6	O	ND	ND
	7	O	ND	12
	9	O	ND	ND
	18	L	ND	ND
	21	L	ND	5
	22	L	ND	ND
	23	L	ND	ND
	26	P	ND	8
	36	O	ND	14
	40	O	ND	ND
	103	O	ND	12
	110	O	ND	12
	Stone	L	ND	ND
	X	O	ND	ND

[a] Data for soils collected 8 October 1981. Taken after 7 days incubation at −0.3 to −1.0 bars and 21 C.
[b] L = Lethent silty clay; P = Panoche clay loam; O = Oxalis silty clay.
[c] None detectable.

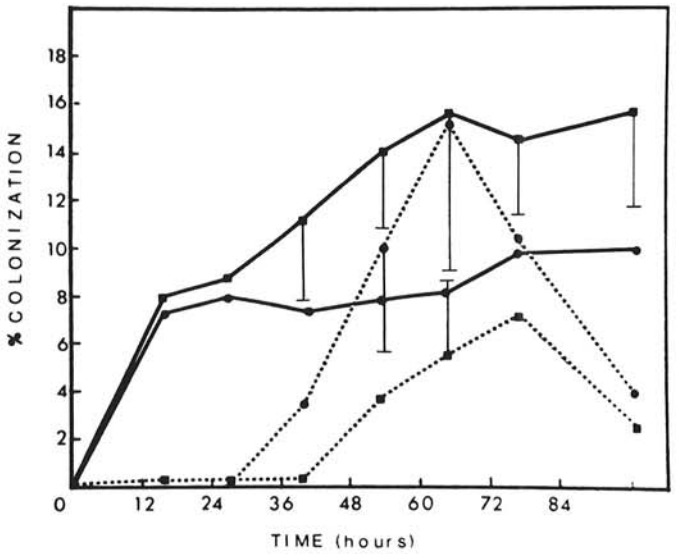

Fig. 1. Effect of NaCl amendment on cotton leaf colonization by *Pythium ultimum* (——) and *P. oligandrum* (----) in a naturally infested, conducive field soil incubated at −0.5 bar and 21 C. Chloride concentration in unamended field soil (■) was 6.2 meq Cl^- per liter, whereas NaCl amended soil (•) had 44.2 meq Cl^- per liter. Bars represent $LSD_{0.05}$.

TABLE 2. Chemical analysis of soils collected after cotton harvest[a]

Soil classification	Site	pH[b]	EC (mmho/cm)	Na^{+c} (meq/100 g)	SO_4^{-2d} (meq/L)
Conducive	3	7.5	2.6	2.1	4.5
	4	7.7	2.0	1.6	6.8
	10	7.5	1.0	0.6	0.6
	12	7.9	1.3	2.7	1.7
	13	7.6	1.3	2.1	2.7
	14	7.6	0.6	0.5	0.3
	27	7.3	2.2	0.6	1.2
	30	7.5	1.2	0.5	0.7
	31	7.3	1.3	0.5	0.7
	32	7.7	1.1	2.1	3.6
	33	7.5	1.5	0.7	1.1
	Mean	···	1.5	1.3	2.3
	SD[e]	···	0.6	0.3	2.1
Moderately suppressive	15	7.5	1.2	0.7	0.1
	25	7.6	1.1	1.4	0.2
	35	7.1	1.8	1.0	···
	100	7.4	1.0	0.6	1.8
	Mean	···	1.3	0.9	1.1
	SD	···	0.4	0.4	0.9
Suppressive	6	7.5	2.6	1.7	6.2
	7	7.5	1.4	0.9	1.4
	9	7.6	1.5	1.2	1.1
	18	7.7	2.5	3.5	6.2
	21	7.8	1.2	1.5	1.8
	22	7.8	3.2	5.3	8.5
	23	7.7	1.9	2.2	5.1
	26	7.3	3.4	1.8	9.1
	36	7.5	1.4	1.6	1.4
	40	7.4	0.7	0.6	1.0
	103	7.6	3.0	2.6	4.4
	110	7.3	1.7	0.8	1.1
	Stone	7.2	3.4	1.4	12.6
	X	7.8	3.0	4.5	7.4
	Mean	···	2.2	2.1	4.8
	SD	···	0.9	1.4	3.7

[a] Soils collected from the furrow shoulder at a depth of 0–12 cm on 10 October 1981.
[b] pH measured in saturation paste.
[c] Exchangeable sodium from ammonium acetate extract.
[d] Soluble sulfate from saturation extract.
[e] SD = Standard deviation.

associated with reduced saprophytic activity of the fungus. When leaf debris colonization was observed on a daily basis for 4 days, the suppression was detected 40 hr after soil amendment (Fig. 1). Suppression of organic matter colonization coincided with an increase in colonization by other primary colonizing fungi, in particular *P. oligandrum*. In NaCl amended conducive soils there

TABLE 3. Chloride concentrations in the saturation extract of field soils sampled after cotton harvest[a]

		Chloride concentrations (meq/L)		
		Collection date		
Suppressive classification	Site	1980	1981	1982
Conducive	3	···[b]	11.3	···
	4	···	6.4	···
	10	···	6.1	···
	12	2.5	7.1	18.8
	13	6.0	4.5	16.9
	14	···	2.9	···
	27	···	10.2	···
	30	···	5.4	···
	31	···	5.0	12.4
	32	···	2.4	···
	33	7.4	5.9	9.1
	Britz	10.4	···	···
Moderately suppressive	5	···	···	8.2
	15	···	5.5	3.0
	25	···	5.3	···
	35	···	8.2	···
	39	13.9	···	12.2
	100	···	4.6	7.9
	102	···	5.5	9.5
Suppressive	2	34.6	···	21.4
	6	···	11.1	16.4
	7	···	6.2	7.5
	9	···	7.7	9.7
	18	···	11.9	20.9
	19	···	···	30.9
	21	25.7	5.9	···
	22	55.6	19.0	···
	23	···	10.6	···
	24	46.6	···	28.5
	26	···	12.6	···
	36	15.2	6.3	···
	37	21.1	···	···
	40	···	2.6	8.3
	41	18.5	···	···
	103	···	13.2	···
	110	···	6.1	···
	Stone	···	13.7	···
	Boston	33.3	···	16.0
	X	···	15.0	19.4

[a] Soils collected on 14 October 1980 and 25 October 1982 were sampled from 0–5 cm at the furrow shoulder, while soils collected on 8 October 1981 were sampled from 0–12 cm at the furrow shoulder.
[b] Fields were not cropped to cotton and were not surveyed.

TABLE 4. Effect of the sodium salts of Cl^-, PO_4, and SO_4^{-2} on net increases in inoculum densities of *Pythium ultimum*[a]

Treatment	Inoculum density (p/g soil)	Unamended control (%)
Control	317 z	100
NaCl[b]	158 y	50
NaH_2PO_4	355 z	112
Control	417 z	100
Na_2SO_4	454 z	109

[a] After 7 days incubation with cotton leaf debris at −0.1 bar. Data in each column for each experiment followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] [Cl^-] was 56.6 meq/L for NaCl-amended soil; [Na^+] of 39.0 meq/L for NaH_2PO_4-amended soil; and [SO_4^{-2}] of 36.5 meq/L for Na_2SO_4-amended soil.

was a 36% reduction in colonization by *P. ultimum*, whereas there was an increase in colonization by *P. oligandrum* from 7 to 15%. The onset of colonization by *P. oligandrum* also occurred sooner in chloride-amended soils compared with the unamended control (40 hr vs. 53 hr, respectively).

Colonization of leaf debris by primary colonizing fungi. *P. ultimum* was present in all soils, but had a low frequency of leaf debris colonization in suppressive soils (Table 5). In these soils, most of the added organic matter was colonized by other primary colonizing fungi, most notable of which was *P. oligandrum*. The frequency of colonization by this fungus in suppressive soils ranged from 27 to 85% of leaf fragments added to the soil, whereas in conducive soils a maximum of 13% colonization was observed. In moderately suppressive soils, the frequency of leaf debris colonization by *P. oligandrum* also was low, with a maximum of 16%. The colonization of leaf debris by other primary colonizing fungi varied from 0 to 38% in suppressive soils and 0 to 33% in conducive soils. In conducive soils, *P. ultimum* was the predominant primary colonizer isolated. The frequency of co-colonization of leaf fragments by *P. ultimum* and *P. oligandrum* was less than 2%, and there was a negative correlation ($r = -0.56$, $P = 0.05$, and slope $= -0.64$) between colonization by *P. ultimum* and *P. oligandrum*. There was a positive correlation between the frequency of leaf colonization by *P. ultimum* and increases in inoculum density (Fig. 2).

TABLE 5. Colonization of cotton leaf debris by fungi in conducive and suppressive soils[a]

Soil classification	Site	Colonization (%)		
		P. ultimum	*P. oligandrum*	Other fungi[b]
Conducive	1[c]	85.9	0.0	0.0
	33	27.4	10.4	12.7
	Britz	11.4	13.4	32.5
Moderately suppressive	2	7.4	3.3	1.5
	14	14.1	15.6	7.3
Suppressive	7	0.7	62.2	38.4
	24	0.7	27.4	0.7
	26	0.7	70.1	6.6
	36	4.4	33.6	7.3
	41	1.5	38.5	12.9
	Boston	1.5	85.2	16.6
	Stone	0.7	50.4	0.0

[a]Soils incubated at −0.1 bar and 21 C.
[b]Unidentified primary colonizing fungi including several other *Pythium* spp.
[c]All soils cropped to cotton except site 1.

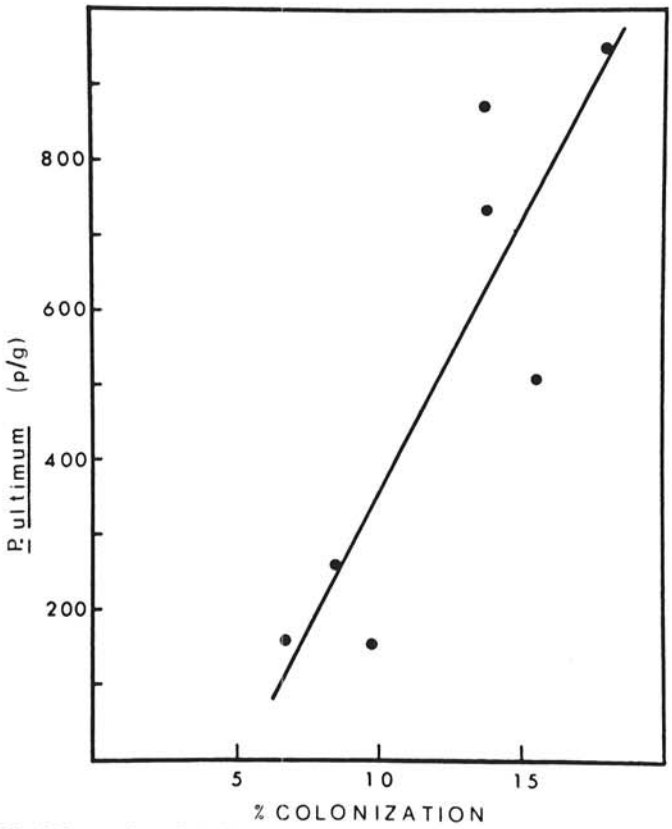

Fig. 2. Regression of the frequency of cotton leaf colonization by *Pythium ultimum* and subsequent increases in inoculum densities. Soils incubated at −0.1 bar at 21 C for 7 days after amendment with leaf debris. ($r = 0.86$, $P = 0.01$, slope = 71.7)

Effect of chloride on leaf colonization. In naturally infested field soil, *P. oligandrum* was more tolerant of elevated chloride concentrations than *P. ultimum* (Fig. 3). Significant reductions in leaf colonization by *P. oligandrum* compared with *P. ultimum* did not occur until chloride concentrations exceeded 133 meq/L, where there was more than a 50% reduction in the frequency of colonization by *P. ultimum*. The chloride concentration at which colonization by *P. oligandrum* was reduced 50% (220 meq/L) was slightly greater than the concentration at which colonization by *P. ultimum* was inhibited by 75% (215 meq/L). At the concentration found in suppressive soils (up to 50 meq/L), chloride had no inhibitory effect on *P. oligandrum*, but reduced the saprophytic activity of *P. ultimum* by 30%.

In autoclaved soil reinfested with *P. oligandrum*, the rate of leaf debris colonization was not affected by additional chloride, whereas in autoclaved soil reinfested with *P. ultimum*, chloride amendments delayed the rate of colonization (Fig. 4). This delay was manifested within 24 hr after soil amendment with leaf debris. The frequency of leaf debris colonization increased with time and gradually approached the value observed for the chloride-unamended control after 84 hr. This was in contrast to the pattern of colonization by *P. ultimum* in chloride-unamended soil, which reached a plateau after 48 hr.

Amendment of conducive field soil with different chloride salts caused significant reductions of saprophytic inoculum increases of *P. ultimum*. At 20 meq of Cl^- per liter, NH_4Cl was the most inhibitory of the salts tested against *P. ultimum* causing an average 66% reduction in saprophytic activity compared with the chloride unamended controls. This was followed in decreasing order of inhibition by $CaCl_2$, KCl, and NaCl (60, 53, and 34%, respectively). When present at 120 meq of Cl^- per liter, only NH_4Cl caused any significant suppression (35%) in the frequency of leaf colonization by *P. oligandrum*, whereas the other chloride salts had no effect.

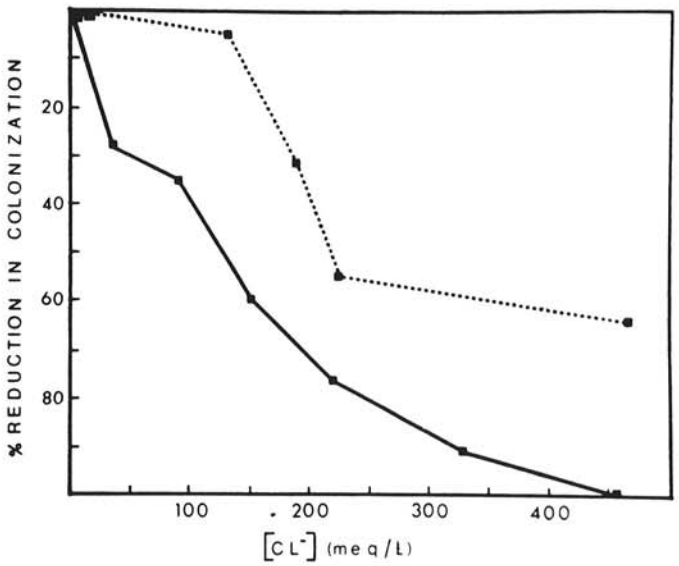

Fig. 3. The effect of increasing Cl^- concentrations on the frequency of cotton leaf colonization by *Pythium ultimum* (——) and *P. oligandrum* (----) in field soils incubated at −0.1 bar and 21 C.

Recovery of *Pythium* spp. on the differential medium. *P. oligandrum* and *P. ultimum* both have characteristic colony morphologies on the differential medium. After 48 hr at 30 C, colonies of *P. oligandrum* were approximately 18 mm in diameter, with diffuse, regularly branched mycelium radiating from the center (Fig. 5). A pigment-free zone was found in the center of the colony surrounded by a dense red zone in which the rose bengal was concentrated. Colony morphologies of *P. ultimum* differed in that they were about 40 mm in diameter after 48 hr and colonies grew from propagules as several thick hyphae covered with short, densely branched hyphae in the center giving way to longer branches at the periphery of the colony (Fig. 6). The mycelium concentrated the rose bengal and had a denser red color than the surrounding medium, especially in the center of colonies. In cases where the soil had not been evenly distributed, or more than 0.04 g of soil per plate was used, the characteristic color differences were not always apparent and colony morphology alone was used to identify species of *Pythium*. When assaying soils with high inoculum densities and using low amounts of soil in dilution plating (e.g., 0.5 g per 10 ml), a rose bengal concentration of 25 μg/ml provided more accurate estimations of inoculum density.

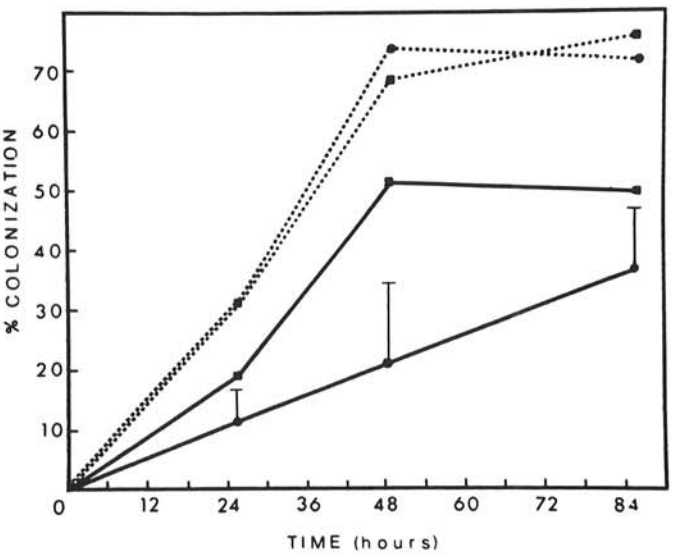

Fig 4. The effect of Cl^- on cotton leaf colonization by *Pythium ultimum* (——) or *P. oligandrum* (----) in autoclaved, artificially reinfested conducive soil incubated at −0.1 bar and 21 C. Chloride-unamended soil (■) contained 3.46–3.71 meq Cl^- per liter whereas the Cl^- amended soil (•) infested with *P. ultimum* or *P. oligandrum* contained 120.6 or 111.4 meq Cl^- per liter, respectively. Bars represent $LSD_{0.05}$.

There was a significant correlation ($P = 0.01$) between the soil drop assay and differential medium for the estimation of inoculum density *P. ultimum* in field soil (Fig. 7). The differential medium, however, yielded slightly lower inoculum densities than the soil drop assay.

Propagule densities of *P. oligandrum* in field soil. There was a trend of greater propagule densities of *P. oligandrum* in suppressive than conducive soils. For example, in both 1981 and 1982 the suppressive soils collectively had mean propagule densities twofold greater than conducive or moderately suppressive soils (Table 6). However, there were several exceptions in the direct association between suppressiveness and propagule densities of *P. oligandrum* at individual field sites (Table 6).

Effects of increased propagule density of *P. oligandrum* on leaf colonization. With increased propagule densities of *P. oligandrum* in a conducive soil there was a decrease in the frequency of colonization (Fig. 8) and saprophytic inoculum increases by *P. ultimum* (Table 4). In soil from site 12 (with an initial inoculum density of *P. ultimum* of 17 propagules per gram of soil), an increase in propagule density of *P. oligandrum* from 13 to 43 propagules per gram of soil caused 47% less colonization after 96 hr and 32% less subsequent increases in inoculum of *P. ultimum* (737 propagules per gram of soil vs. 498 propagules per gram of soil, respectively). Similar effects were observed with soil from site

Fig. 5. Colony morphology of *Pythium oligandrum* (Po) from soil dilution plate after 48 hr at 21 C on differential media for *P. oligandrum*.

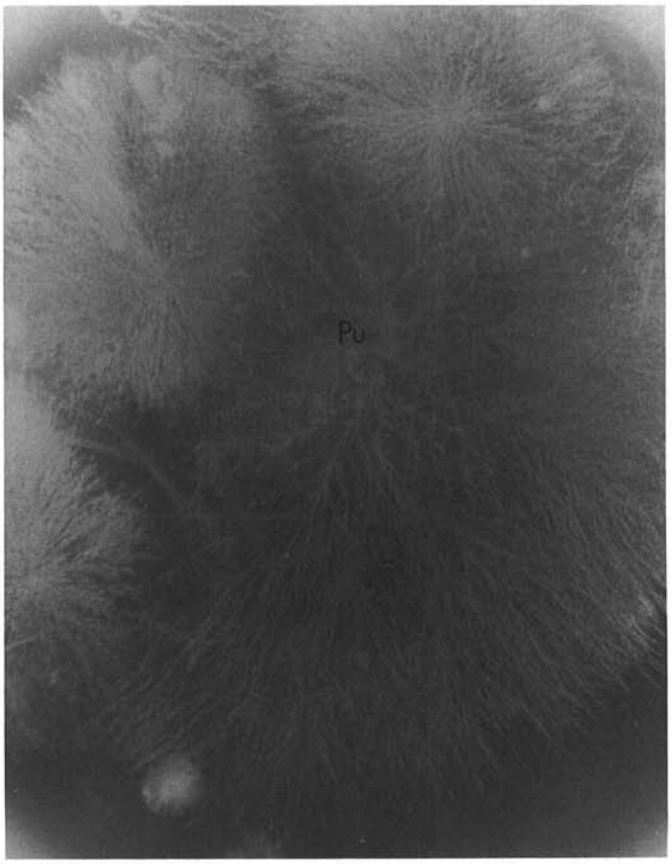

Fig. 6. Colony morphology of *Pythium ultimum* (Pu) from soil dilution plate after 48 hr at 21 C on differential media for *P. oligandrum*.

33 (with nondetectable levels of *P. ultimum*) when the propagule density of *P. oligandrum* was increased from 2 to 24 propagules per gram of soil: Colonization by *P. ultimum* was 60% less and subsequent inoculum increases less (164 propagules per gram of soil vs. 56 propagules per gram, respectively). With both soils, the lower rates of colonization by *P. ultimum* was manifested 24 hr after soil amendment with leaf debris and coincided with an increased frequency of leaf colonization (1,690% for soil 12 and 671% for soil 33) by *P. oligandrum* (Fig. 8).

Saprophytic increases in inoculum of *P. ultimum* were inhibited further by increasing the propagule densities of *P. oligandrum* (Fig. 9). With site 12, which had an initial inoculum density of *P. ultimum* of 50 propagules per gram of soil, the addition of 100 propagules per gram of soil of *P. oligandrum* suppressed net inoculum increases of *P. ultimum* by 34%, allowing a net increase of 1,163 propagules per gram of soil after leaf amendment compared with 1,750 propagules per gram of soil for *P. oligandrum* uninfested soil. Whereas for site 33 (initial inoculum density of *P. ultimum* of 17 propagules per gram of soil), the addition of 100 propagules per gram of soil of *P. oligandrum* suppressed net increases in inoculum density of *P. ultimum* by 64%, with a net increase of 221 propagules per gram of soil compared with 621 propagules per gram of soil for the uninfested control with *P. oligandrum*.

As can be seen from these two sets of experiments, the magnitude of reduction in saprophytic inoculum increases of *P. ultimum* caused by *P. oligandrum* is influenced by the initial inoculum density of *P. ultimum*, e.g., those soils with greater initial inoculum densities of *P. ultimum* are less affected by *P. oligandrum* than soils with lower initial inoculum densities.

TABLE 6. Propagule densities of *Pythium oligandrum* in field soil collected 8 October 1981 and 25 October 1982 from furrow shoulders in fall after cotton harvest

Soil classification	Site	Soil collection date (propagules/g) 1981	1982
Conducive	3	37	...[a]
	4	40	...
	10	18	...
	12	22	13
	13	36	36
	14	11	...
	27	16	...
	30	2	...
	31	3	...
	32	2	...
	33	3	2
Moderately suppressive	15	8	6
	25	27	...
	35	8	...
	39	...	12
	100	...	17
	101	...	19
	102	...	17
Suppressive	2	...	3
	6	27	26
	7	43	30
	9	35	25
	18	15	11
	19	...	56
	21	3	...
	22	16	...
	23	2	...
	24	...	11
	26	87	...
	36	15	...
	37	...	15
	40	5	17
	103	55	...
	Boston	...	162
	Stone	79	...
	X	9	10

[a] Fields were not cropped to cotton and were not surveyed.

Effect of NaCl and *P. oligandrum* on patterns of leaf colonization. By increasing the chloride concentration in conducive field soil to 22 meq/L, there was a slight (not significant) increase in the frequency of leaf colonization by *P. oligandrum* and less colonization by *P. ultimum* (Table 7). Soil amendment with 50 propagules per gram of soil of *P. oligandrum* caused a significant increase in the frequency of colonization by this fungus and, consequently, less colonization by *P. ultimum*.

Soil amendments with both chloride and *P. oligandrum* had a greater increase in colonization by *P. oligandrum* than observed for either treatment individually (Table 8). However, colonization by *P. ultimum* was only slightly below that observed for the amendment with *P. oligandrum* alone (not significant). Because of a large variability in the inoculum increases of *P. ultimum* among experiments, differences in the mean values are not significantly different ($P = 0.05$), but the data reflect relative changes in the frequency of leaf colonization by *P. ultimum* in response to chloride and *P. oligandrum*, with the greatest suppression in colonization observed for the combined treatments.

Converting a suppressive soil to an conducive soil. During the first week of incubation with leaf debris in a suppressive soil, the frequency of leaf colonization by *P. ultimum* was lower (0.7%) compared with the frequency of leaf colonization by *P. oligandrum* (36%) (Table 9). Inoculum density of *P. ultimum* was undetectable and, on the basis of the first leaf amendment, this soil met the criterion of a suppressive soil. However, after the second leaf amendment, the frequency of colonization by *P. ultimum* increased to 7% with a slight decrease in colonization by *P. oligandrum* to 33%. After the second amendment, the inoculum density of *P. ultimum* was 45 propagules per gram of soil and, thus, the soil met the criterion of a moderately suppressive soil. There was a further increase in the frequency of colonization by *P. ultimum* (29%) after the third amendment, whereas colonization by *P. oligandrum* was reduced to 16%. After three weekly

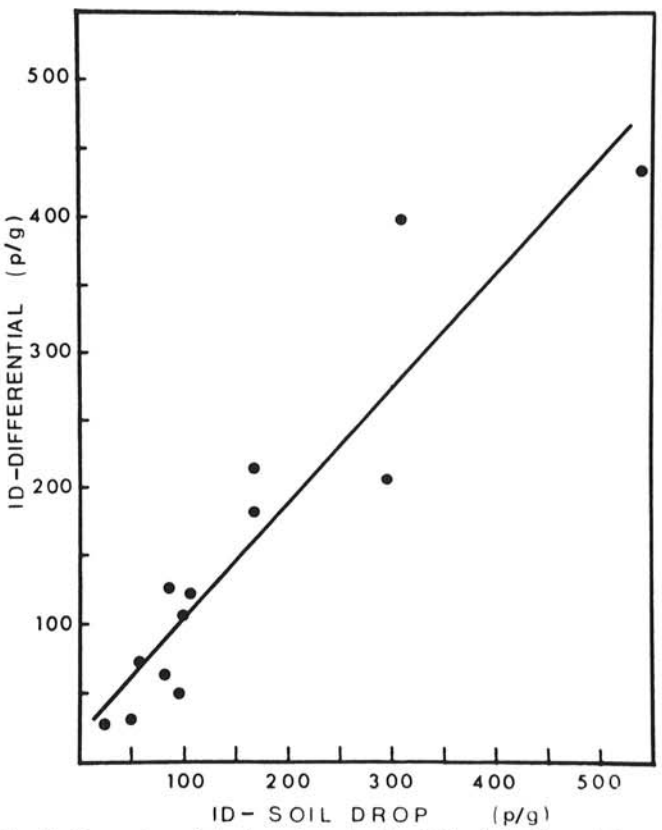

Fig. 7. Regression of the inoculum density (ID) of *Pythium ultimum* determined by the soil drop assay and dilution plating on differential medium ($r = 0.93$, $P = 0.01$, slope = 0.84).

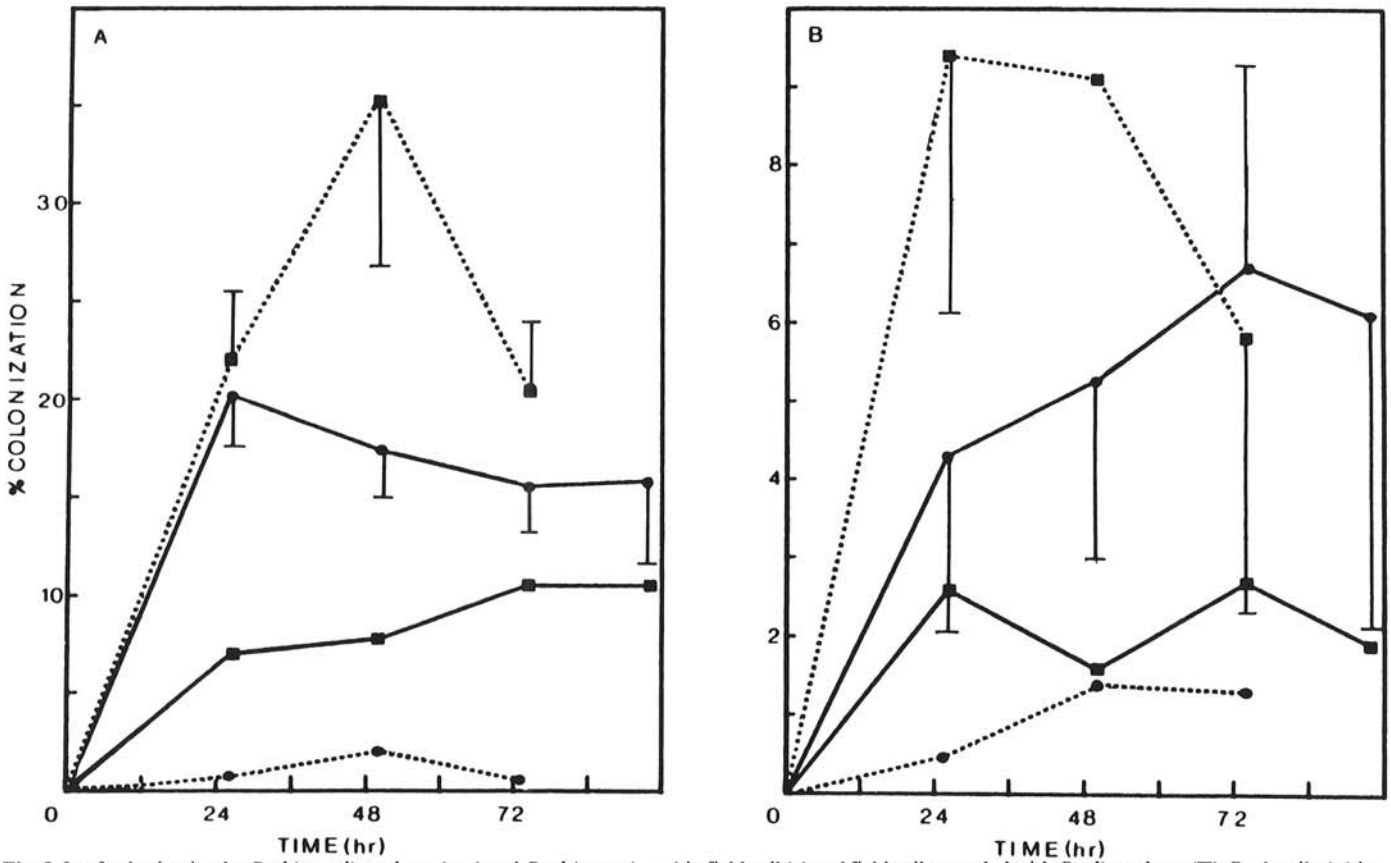

Fig. 8. Leaf colonization by *Pythium oligandrum* (----) and *P. ultimum* (——) in field soil (•) and field soil amended with *P. oligandrum* (■). Both soils A (site 12) and B (site 33) are conducive soils maintained at −0.1 bar at 21 C. Initial propagule densities of *P. ultimum* and *P. oligandrum* in soil A were 17 propagules per gram of soil and 43 propagules per gram of soil, respectively, and in soil B were undetectable (less than 8 propagules per gram of soil) and 24 propagules per gram of soil, respectively. Bars represent $LSD_{0.05}$.

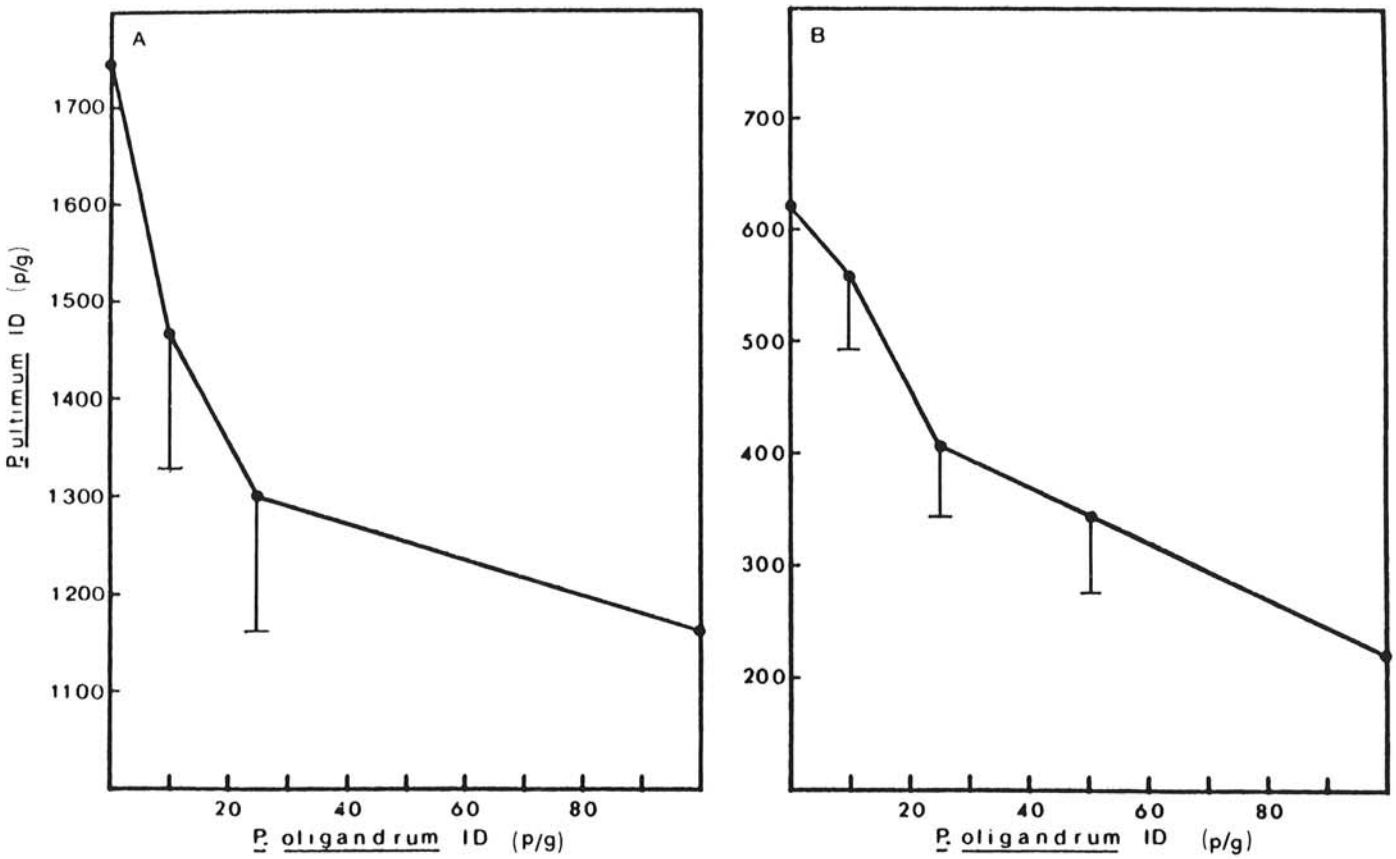

Fig. 9. Effect of amending conducive soils with oospores of *Pythium oligandrum* on saprophytic inoculum increases of *P. ultimum* after cotton leaf amendment and incubation at −0.1 bar and 21 C for 7 days. Soil A (site 12) had an initial inoculum density of *P. ultimum* of 50 propagules per gram of soil and soil B (site 33) had 17 propagules per gram of soil. Bars represent $LSD_{0.05}$.

amendments of cotton leaf debris, the soil met the criterion of a conducive soil, as inoculum densities of *P. ultimum* had increased to 431 propagules per gram of soil.

DISCUSSION

The suppressiveness of certain soils in the San Joaquin Valley to saprophytic inoculum increases of *P. ultimum* was a reasonably stable phenomenon, e.g., soils collected in 1981 had similar suppressive ratings as those collected in 1977 (18). Several of the collection sites (Boston, Stone) were surveyed since 1972 (17) and have remained suppressive since the initial investigation. Thus, although the pathogen was present in field soils, suppressiveness was retained when the common rotation practices (cotton, barley, fallow) of the region were followed.

As reported previously (18), the finer textured Lethent silty clay loams and Oxalis silty clay soils tended to be suppressive, and the coarser textured Panoche clay loam soils tended to be conducive. Relationships between soil texture and pathogen suppressiveness have been observed with other suppressive soils as well (2,28,29, 34,39). Although there were strong associations between suppressiveness to *P. ultimum* and soil texture, this alone was not responsible for the reduced saprophytic activity of *P. ultimum* in suppressive soils. Several finely textured soils were conducive to *P. ultimum* (Table 1) and amending suppressive soils with varying dilutions of sand did not alleviate suppressiveness (18). Because leaching of suppressive soils occasionally relieved suppressiveness, it appeared that soil mineral salts were involved in inhibiting *P. ultimum* (18).

In the cultivation of cotton in the San Joaquin Valley, irrigation is halted in the late summer several months before the fall harvest. With the cessation of irrigation, salts are carried up through the soil profile by capillary action and concentrated at the surface by evaporation. Chemical composition at the soil surface is important because this is where organic residues are deposited and *P. ultimum* is most active as a saprophyte (17).

Chemical analysis of the survey soils after fall harvest revealed that suppressive soils tended to have greater concentrations of sodium, sulfate, and chloride than conducive soils (Tables 2 and 3). However, of these ions, only chloride inhibited the saprophytic inoculum increases of *P. ultimum* when present in concentrations approximating those found in suppressive soils (Table 4). This was caused by a reduced frequency of colonization of organic matter by *P. ultimum* manifested soon after residue amendment of the soil. This reduction coincided with an increase in the level of colonization by another primary colonizing fungus, *P. oligandrum* (Fig. 1). *P. oligandrum* is more tolerant of chloride than *P. ultimum* and therefore has a greater saprophytic activity than *P. ultimum* in soils with greater chloride concentrations (Fig. 3).

From investigations of organic matter colonization in soils from the collection sites, it was observed that *P. oligandrum* was a common soil saprophyte and the most successful pioneer saprophyte retrieved from suppressive soils (Table 5). Although *P. ultimum* was present in these soils, it accounted for a small fraction of the fungi retrieved from added leaf debris and consequently did not increase saprophytically. This was in contrast to the behavior of *P. ultimum* in conductive soils. With low levels of competition from other primary colonizing fungi (as observed by the leaf colonization assay, Table 5), *P. ultimum* was the predominant saprophyte isolated, even when present at low inoculum densities (Table 1). Because there is a significant correlation between the frequency of leaf colonization and subsequent increases in inocula of *P. ultimum* (Fig. 3), greater increases in inoculum densities of *P. ultimum* occurred in conducive soils than in suppressive soils.

The greater levels of leaf debris colonization by *P. oligandrum* in suppressive soils is a reflection of the relative propagule densities of this fungus, with suppressive soils tending to have greater propagule densities than conducive soils (Table 6). The influence this would have on the saprophytic activity of *P. ultimum* was demonstrated by artificially increasing the levels of *P. oligandrum* in conducive soils. An inverse relationship was observed between subsequent saprophytic increases in the inoculum density of *P. ultimum* and the propagule density of *P. oligandrum* added to the soil (Fig. 9). This was due to enhanced competition for substrate soon after its amendment to field soil (Fig. 8). The effect of increased propagule densities of *P. oligandrum* on reducing the saprophytic inoculum increases of *P. ultimum* was more dramatic in soils with initially lower inoculum densities of *P. ultimum* than soils with higher inoculum densities. The inoculum dependent relationship between *P. ultimum* and antagonists concurs with the

TABLE 7. Effect of supplements on propagules of *Pythium oligandrum* on saprophytic increases in inoculum density by *Pythium ultimum*[a]

Soil	*P. ultimum* (p/g soil)	Control (%)
12[b]	737	100
12 + *P. oligandrum*[c]	499	68
33[b]	163	100
33 + *P. oligandrum*[d]	56	34

[a] Data collected after 7 days incubation at −0.1 bar. Data in each column for each soil are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Raw conducive field soil. Initial inoculum densities of *P. ultimum* were 17 propagules per gram for soil 12 and undetectable (less than 8 propagules per gram) for soil 33.
[c] Amended to 43 propagules per gram of soil with oospores of *P. oligandrum*.
[d] Amended to 24 propagules per gram of soil with oospores of *P. oligandrum*.

TABLE 8. The influence of elevated chloride concentrations and propagule densities of *P. oligandrum* in a conducive soil on the colonization of leaf debris and subsequent increases in inoculum density of *Pythium ultimum*[a]

	Soil treatment			
Pythium spp.	Control	NaCl[b]	*P. oligandrum*[c]	NaCl + *P. oligandrum*
Colonization %[d]				
P. oligandrum	1.8 x	3.0 x	9.7 y	14.7 z
P. ultimum	20.4 z	18.4 zy	14.8 y	13.8 y
Inoculum density (p/g)[e]				
P. ultimum	402.7 z	363.0 z	349.1 z	233.3 z

[a] Data reflect the mean of three experiments. Data followed by the same letter in each row are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] Initial chloride concentrations were approximately 6 meq/L. Chloride-amended soils were saturated with 0.03 M NaCl before equilibration on tension plate, giving final chloride concentration of approximately 22 meq/L.
[c] Field soil amended to give 50 propagules per gram of soil *P. oligandrum*.
[d] Greatest level of leaf debris colonization recorded during 7 days of incubation.
[e] Inoculum density after 7 days of incubation at −0.1 bar and 21 C. Initial inoculum density was 25 propagules per gram.

TABLE 9. Effect of amending a suppressive soil on a weekly basis with cotton leaf debris (0.25 g per 75 g of soil) on the patterns of leaf colonization of *Pythium oligandrum* and *P. ultimum* and subsequent increases in the inoculum density of *P. ultimum*[a]

	Colonization (%)		Inoculum density (p/g soil)
Week	*P. ultimum*	*P. oligandrum*	*P. ultimum*
1	0.7 z	36.2 z	ND[b] z
2	7.3 y	32.7 z	45 y
3	28.9 x	16.2 y	431 x

[a] After 7 days incubation between −0.1 and −0.3 bar in a moist chamber at 21 C. Data followed by the same letter in each column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] ND = None detected.

observations of Bouhot (6) on the reduced effectiveness of *Actinomucor elegans* in suppressing the saprophytic activity of *Pythium* spp. in the soil as pathogen inoculum densities were increased.

Thus, it appears that the low levels of saprophytic inoculum increases of *P. ultimum* observed in suppressive soils may be caused by an ecological interaction with another pioneer saprophyte, *P. oligandrum*. With the low inoculum densities of *P. ultimum* in suppressive soils, *P. oligandrum* is able to effectively outcompete *P. ultimum* for organic matter colonization. Because prior colonization by *P. oligandrum* apparently precludes subsequent colonization by *P. ultimum* [as reflected by reductions in the frequency of colonization of *P. ultimum* with increasing propagule densities of *P. oligandrum*. (Figs. 8 and 9) and the low level of co-colonization of leaf debris by both fungi], saprophytic inoculum increases of *P. ultimum* are restricted. The ecological balance between these two fungi is further shifted to favor *P. oligandrum* by the greater chloride concentrations in suppressive soil (Table 3). Barton (4,5) and Hine and Trujillo (20) observed a similar intolerance to competition for substrate colonization with *P. mamillatum* Meurs. and *P. aphanidermatum* (Edson) Fitzpatrick, respectively, when substrates were previously exposed to other colonizers. Because of the continued inability of *P. mamillatum* to colonize previously colonized substrates even after fumigation and addition of fresh nutrients, Barton (5) proposed that diffusable metabolic by-products of other primary colonizers, not just nutrient availability, were responsible for inhibition of the saprophytic activity.

The mechanism by which chloride inhibits the saprophytic activity of *P. ultimum* is unclear. However, because the degree of leaf colonization in autoclaved reinfested soil amended with chloride approached the values of unamended controls with time (Fig. 4), chloride may delay germination and/or vegetative growth. Although chloride salt amendments increase the osmotic potential of the soil solution, it is unlikely that this could account for the inhibition of growth or germination of *P. ultimum* unless concentrations were higher than those observed in suppressive soils. While NaCl at concentrations of 56 meq of Cl^- per liter caused a 50% reduction in inoculum increases, Na_2SO_4 at 36 meq SO_4^{-2} per liter had no effect (Table 4). The osmotic potentials of these concentrations are −2.6 and −2.2 bars, respectively (19). Other experiments (F. N. Martin, *unpublished*) with about 90 meq of Na^+ per liter as NaH_2PO_4 also had no effect on the saprophytic activity of *P. ultimum* (osmotic potential of approximately −5 bars). Therefore, reductions in the saprophytic activity of *P. ultimum* associated with chloride amendments are more likely to be due to chloride toxicity than to osmotic effects on the fungus.

Of the different chloride salts tested (20 meq of Cl^- per liter) for their effect on *P. ultimum* (sodium, potassium, ammonium, and calcium), ammonium chloride was the most inhibitory. This salt was also the only one tested inhibitory to *P. oligandrum* (at 120 meq of Cl^- per liter), thereby supporting the observation that *Pythium* spp. may be especially sensitive to ammonia or ammonium. Chun and Lockwood (10) noted that survival of sporangia of *P. ultimum* was reduced in soils where an excess of ammonia was released following urea amendments, as did Lewis and Lumsden (21) with soil amendments of CaO. Ammonia also reduced the survival of oospores of *P. aphanidermatum* in field soil (21,33) and inhibited spore germination and reduced survival of several *Phytophthora* spp. (36,41).

P. oligandrum has been reported to be mycoparasitic on a number of phytopathogenic fungi (3,12,13,37) and an effective biological control agent for damping-off caused by *P. ultimum* (3,26,38). Mycoparasitism is observed in dual culture and also may occur on crop debris in the soil. However, isolations from field soils revealed a low frequency of leaf debris colonized by both fungi, even from soils with high propagule densities and individual frequencies of colonization. If mycoparasitism was significantly reducing the recovery of *P. ultimum*, a greater incidence of co-colonized leaf fragments would be expected, as would reductions in the frequency of isolation and/or inoculum increases of *P. ultimum* over time. This was not observed. Therefore, although mycoparasitism may occur in co-colonized leaf debris, it does not appear to be primarily responsible for reductions in leaf colonization by *P. ultimum* in soils amended with *P. oligandrum*. Rather, reductions in recovery of *P. ultimum* appear to be the result of prior colonization of organic substrates by *P. oligandrum*, thereby preventing establishment of *P. ultimum*.

The ecological balance between *P. oligandrum* and *P. ultimum* in suppressive soils can be shifted to favor *P. ultimum* by increasing its inoculum density. This causes an increase in the frequency of colonization and subsequent inoculum increases by *P. ultimum*. A suppressive soil was converted to a conducive soil by this procedure (F. N. Martin, *unpublished*). Weekly amendments with cotton leaf debris for 3 wk also converted a suppressive soil to a conducive soil (Table 9) (18). With each successive amendment the frequency of colonization by *P. oligandrum* decreased while colonization by *P. ultimum* increased. Thus, it appears as though the maintenance of soil suppressiveness may be an inoculum dependent phenomenon. Once the inoculum density of *P. ultimum* is above a certain level, the combined influences of *P. oligandrum* and chloride no longer significantly suppress the saprophytic activity of the pathogen. Additional evidence for this is the observation that the ability of *P. oligandrum* to reduce the saprophytic activity of *P. ultimum* is lower in soils with greater inoculum densities of *P. ultimum* (Fig. 9) and also may be the reason why the combined influences of chloride and *P. oligandrum* in a conducive soil did not completely inhibit the saprophytic activity of *P. ultimum* (Table 8). Because the frequency of colonization and inoculum increases of *P. ultimum* increased with successive leaf amendments, it appears that under the environmental conditions used *P. ultimum* is a better competitor than *P. oligandrum*.

The capacity of *P. ultimum* to overcome competition by *P. oligandrum* at high chloride concentrations during repeated organic matter amendment may explain why certain finely textured soils with high propagule densities of *P. oligandrum* and chloride concentrations were conducive. Although cropping histories of these sites are incomplete before 1980, some of them (sites 3 and 4) were the only sites surveyed that included sugar beets in the normal cotton, barley, fallow rotation. The quantities of organic residues returned to soil after sugar beet cropping are substantial, which may alter the suppressive nature of these soils in much the same way as repeated artificial amendments.

The relationship between levels of saprophytic competition and the saprophytic/pathogenic activity of a *Pythium* spp. has been observed by other investigators. In studies of Pythium suppressive soils in France, Bouhot et al (6,7) observed a relationship between the ability of a *Pythium* spp. to colonize organic substrates and the severity of damping-off. In field soils with greater numbers of other primary colonizing fungi (mostly members of the Mucorales), there was greater competition for substrate and the incidence of disease was low. These soils were termed suppressive. Thus, for each soil, suppressiveness to disease was a function of the competitive saprophytic ability of the *Pythium* spp. Factors that reduced its competitive saprophytic ability such as direct soil amendment with *A. elegans*, enhanced suppressiveness in conducive soils (6). Lifshitz et al (22,23) also observed an inverse relationship for saprophytic inoculum increases between two *Pythium* spp. mediated by temperature and pH.

Nutrient competition was suggested to account for reductions in pathogenic activity of *P. ultimum* by Watson (40). After soil amendments with lettuce debris, the inoculum density of *P. ultimum* increased, but the inoculum potential of these propagules eventually decreased with time. Because the inoculum densities of *P. ultimum* remained unchanged, Watson (40) attributed the reduction in inoculum potentials to enhanced competition from other soil microbes stimulated by organic matter amendments. Similar mechanisms of enhanced competition for nutrients have been postulated to reduce the inoculum potential of *R. solani* (16,31) and to be involved in Fusarium suppressive soils (1,2,24). In the current study, competition for nutrients occurred before or during organic matter colonization and reproduction by *P. ultimum*.

On the basis of the results of this and previous studies (17,18), we believe that soil suppressiveness to *P. ultimum* in certain regions of the San Joaquin Valley is a dynamic phenomenon and dependent on both the physical and biological characteristics of the soil and the agricultural practices (e.g., irrigation, cropping, and cultural activities) of the region that affect the competitive saprophytic ability of this fungus. Specifically, we conclude that: 1) soil suppressiveness to *P. ultimum* is caused by periodic increases in salinity when chloride concentrations are raised to levels that inhibit its saprophytic activities, 2) high concentrations of chloride favor the competitive saprophytic ability of *P. oligandrum*, a phenomenon that allows the build-up of soil populations of *P. oligandrum* at the expense of *P. ultimum*, 3) the occurrence of suppressiveness to *P. ultimum* is dependent on the inoculum density of this fungus, e.g., suppressiveness is relieved once pathogen inoculum densities are raised above certain levels, whether this be achieved by artificial addition of propagules or by repeated addition of organic substrates to suppressive soils, and 4) the association of soil textural types previously noted to be closely associated with suppressiveness to *P. ultimum* is related to the proneness of the soil type to salinity problems.

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