Nature of Suppression of *Pythium splendens* in a Pasture Soil in South Kohala, Hawaii

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ABSTRACT

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The effect of a pasture soil suppressive to *Pythium splendens* in the South Kohala district on the island of Hawaii on germination or growth of various microorganisms ranged from inhibitory to stimulatory compared with that of conducive soil from Hilo, HI. The soil was suppressive to damping-off caused by *P. splendens* and *P. aphanidermatum*, but not to damping-off caused by *Phytophthora palmivora* and *P. capsici* and it inhibited germination of spores of *P. splendens* and *P. aphanidermatum*, but not those of *P. palmivora* and *P. capsici*. Sterilization by autoclaving or γ-irradiation completely nullified the inhibitory effect of suppressive soil. Inhibition of germination of sporangia of *P. splendens* was restored to sterilized suppressive soil by reinfestation by adding suspensions of either suppressive or conducive soil. Reinfestation of sterilized conducive soil with suppressive soil suspension did not convert it to inhibitory soil. Inhibition of sporangial germination also was restored in autoclaved suppressive soil by reinfestation with actinomycetes, bacteria, or fungi. In mixtures of suppressive and conducive soil, the inhibitory effect of suppressive soil varied inversely with the proportion of conducive soil. Germination inhibition of mixed soil decreased after incubation for 14 and 28 days. Amendment of suppressive soil with 333 ppm of rose bengal, 6,667 ppm of streptomycin, or 3,333 ppm of benomyl partially nullified its inhibition of sporangium germination. The solution obtained from suppressive soil amended with rose bengal or streptomycin was inhibitory to bacteria and actinomycetes but not fungi, whereas the solution obtained from benomyl-amended soil was inhibitory to fungi, but not bacteria and actinomycetes. Neither volatile nor nonvolatile inhibitors were detected in suppressive soil. Suppressive soil remained inhibitory to germination when its pH was adjusted from 6.8 to 5.4, and conducive soil remained conducive to germination when its pH was adjusted from 5.4 to 6.8. Results of this study suggest that a combination of unspecific microbiological factors and unknown abiotic factors is responsible for the microbiological suppressiveness of this pasture soil from South Kohala, HI.

Additional key words: *Fusarium oxysporum*.

Naturally occurring suppressive soils are usually recognized by consistent observation of lower incidence of plant diseases in a certain area in comparison with that in the nearby area (1,2,6). The number of suppressive soils reported so far is very limited. Recently, Ko and Ho (13,14) developed methods for screening soils for suppression of sporangial germination of *Pythium splendens* and mycelial growth of *Rhizoctonia solani*. Soil in a small pasture in the South Kohala district on the island of Hawaii was found to be highly suppressive to germination of sporangia of *P. splendens*. The soil was subsequently found to be suppressive to damping-off of cucumber caused by *P. splendens*.

The purpose of the experiments reported here was to investigate the characteristics and the mechanism of the pathogen suppression in this soil. A brief account of this work was reported (10).

MATERIALS AND METHODS

Soils. Suppressive soil collected from a pasture in South Kohala (very fine sandy loam, pH 6.8) contained about 2.4×10^5 fungal, 19.7×10^6 bacterial, and 4.8×10^6 actinomycete propagules per gram dry weight. Conducive soil collected from a corn field at Hilo (silty clay loam, pH 5.4) contained about 5.7×10^5 fungal, 7.7×10^6 bacterial, and 6.9×10^4 actinomycete propagules per gram dry weight. Soils were collected from depths ranging from 0 to 10 cm after the surface litter was cleared, sieved through a 2-mm screen, adjusted to about -7 bars moisture, and stored in closed glass jars. Unless otherwise stated, they were used within 2 mo.

Microorganisms used in germination, growth, or pathogenicity studies. Sporangia of *Phytophthora palmivora* (Butler) Butler (isolate 110F) and *P. capsici* Leonian (isolate P287) were produced on 10% V-8 juice agar (10% V-8 juice, 0.02% $CaCO_3$, 2% Bacto agar) under light (cool white, 20 lux) for 5–7 days at 24 C. Sporangial suspensions were obtained by spraying the culture plate with distilled water from an atomizer. Zoospores were liberated by incubating the sporangial suspensions at 15 C for 30 min, and separated from sporangia by passing the suspensions through two layers of facial tissue. Zoospores of *Pythium aphanidermatum* (Edson) Fitzpatrick (isolate #606) were obtained according to the method of Rahimian and Banihashemi (18). The method of Ko and Ho (14) was used to obtain sporangia of *P. splendens* Braun (isolate #106F) and *P. ultimum* Trow (isolate #793). Ascospores and conidia of *Calonectria crotalariae* (Loos) Bell & Sobers (isolate #117F) were obtained according to the method of Hwang and Ko (8). *Neurospora tetrasperma* Shear & Dodge (isolate #38F)

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ascospores were obtained as described by Ko and Lockwood (16) and used after heat activation at 58 C for 20 min. Sporangiospores of *Mucor hiemalis* Wehmer (isolate #88F) and *M. ramannianus* Moeller (isolate #41F), conidia of *Alternaria alternata* (Fries) Keissler (isolate #111F), and macroconidia of *Fusarium oxysporum* Schlecht. f. sp. *cubense* (E. F. Smith) Snyder & Hansen (isolate #118F) and *F. oxysporum* Schlecht. f. sp. *lycopersici* (Saccardo) Snyder & Hansen (isolate #119F) were produced by growing the fungi on 10% V-8 juice agar under light (cool white, 20 lux) at 24 C for 7 days. Sclerotia of *Sclerotium rolfsii* Saccardo (isolate #120F) were obtained by growing the fungus on potato-dextrose agar under light (cool white, 20 lux) at 24 C for 14 days, and mycelial disks of *Rhizoctonia solani* Kuhn (isolate #R434) were obtained according to the method of Ko and Ho (14). *Xanthomonas campestris* (Pammel) Dowson (A249-1), *Pseudomonas solanacearum* (Smith) Smith (AG16), *Agrobacterium radiobacter* (Beijerinck and Van Delden) Conn, *Nocardia erythropolis* (Gray and Thornton) Waksman and Henrici isolate #36A), *Streptomyces alboniger* Porter et al (isolate #23A), and *S. scabies* (Thaxter) Waksman and Henrici were grown on 10% V-8 juice agar at 24 C. A loopful of cells or spores from the 2-day-old culture of bacteria or 7-day-old culture of actinomycetes was suspended in 5 ml of sterile distilled water in a test tube. A small amount (~0.1 mg) of Tween-20 on a needle tip was added as a wetting agent to the test tube containing actinomycete spores. Concentrations of spores were determined by the microliter syringe method (11).

Pythium aphanidermatum, P. ultimum, Phytophthora capsici, and *R. solani* were obtained from M. Aragaki, and *F. oxysporum* f. sp. *lycopersici* was obtained from W. Nishijima. Bacteria and actinomycetes were supplied by A. M. Alvarez and J. L. Lockwood, respectively.

Pathogenicity tests. About 150 ml of sporangial suspension of *P. splendens* was thoroughly mixed with 1,200 g of suppressive or conducive soil in a polyethylene bag. Infested soil was distributed in four pots (10 cm), and 15 cucumber seeds (cultivar Lehua No. 1) were planted in each pot. Damping-off of seedlings was recorded after 2, 4, 6, 9, and 10 days. For *P. capsici*, 100 g of soil in a pot was mixed with 5 ml of zoospore suspension and planted with eight tomato seeds (cultivar Healani), and data were recorded after 2 wk. For *P. palmivora*, eight papaya seedlings (cultivar Sunrise Solo, 5–7 cm height) were transplanted to a pot which contained 300 g of soil mixed with 10 ml of zoospore suspension, and data were recorded after 1 mo. For *P. aphanidermatum*, 10 ml of zoospore suspension was poured into 300 g of soil in a pot which was then planted with 16 cucumber seeds. Data were recorded after 10 days. Three inoculum densities were used for *P. splendens* and two were used for each of the other pathogens.

Germination of fungal spores on soils. Since ascospores of *N. tetrasperma* (16) and ascospores and conidia of *C. crotalariae* (8) are not sensitive to general soil fungistasis, they were added to soil without nutrient amendment. For spores of other fungi that are sensitive to general soil fungistasis, nutrients were added to overcome that effect. These spores were suspended in either cucumber root extract or 25% V-8 juice adjusted to pH 6 with 1 N KOH and filtered through Whatman No. 1 filter paper before being placed on soil surface as described by Ko and Ho (14). After incubation at 24 C for 12 hr for sporangiospores of *M. hiemalis* and *M. ramannianus* and 6 hr for spores of the other nine fungi, spores were stained with rose bengal (1% rose bengal, 5% phenol, and 0.01% $CaCl_2$ in distilled water). Germination of *N. tetrasperma* ascospores, *A. alternata* conidia, and *P. splendens* sporangia was counted directly on the soil surface by using a Zeiss Universal Microscope equipped with a Model II C vertical illuminator at the magnification of ×200. Spores of other fungi along with soil particles were scraped off the soil surface with a scalpel, destained in a drop of 5 N NaOH plus 0.5 N NaCl on a glass slide, covered with a coverslip, and observed at ×600 magnification (12).

Growth of microorganisms on soils. Mycelial growth of *R. solani* and *S. rolfsii* on soil was measured according to the method of Ko and Ho (14). For measuring the increase in the numbers of cells of bacteria and actinomycetes, a soil block (50 × 30 × 30 mm) with smoothed surface was made in a petri plate (90 mm). A thin water-agar block (15 × 10 × 1 mm) was placed on a sterile polycarbonate membrane (20 × 20 mm) on the surface of the soil block, and 5 μl of bacterial or actinomycete suspension was pipetted onto the agar block with a Pipetman digital microliter pipet (West Coast Scientific, Inc., Berkeley, CA 94705). After incubation at 24 C for 24 hr, the water agar block was transferred to a glass slide. Microorganisms were stained with cotton blue (100 mg cotton blue in 100 ml of 84% lactic acid), covered with a coverslip, and observed under a compound microscope.

Population densities and isolation of microorganisms in soil. Soil suspensions were prepared by grinding 10 g of soil with 90 ml of sterile distilled water in an Omni mixer operated at 4,500 rpm for 2 min, and serial dilutions (10^{-2}, 10^{-3}, and 10^{-4} for fungi; 10^{-4}, 10^{-5}, and 10^{-6} for actinomycetes; and 10^{-5}, 10^{-6}, and 10^{-7} for bacteria) were plated on selective media. Surfactant-PDA (19), alkaline water agar (7), and PCNB-soil extract agar (4) were used for determining populations of fungi, actinomycetes, and bacteria, respectively.

Soil treatments. Thirty grams of soil in a jar (120 ml) capped with aluminum foil was either autoclaved for 15 min at 121 C or exposed to gamma irradiation at the dosage of 2.5 Mrad. The same amount of soil in a petri plate (90 mm) was steamed in a steamer for 15 min.

To reinfest soil, 3 ml of suppressive or conducive soil suspension (10 g of soil in 90 ml distilled water) was mixed with sterilized soil in a jar. Sterilized soil mixed with sterile distilled water at the same ratio was used as controls. For reinfestation with isolated microorganisms, morphologically distinct colonies from suppressive soil on selective media were isolated. Sixteen fungal, 15 bacterial, and 12 actinomycete isolates were obtained and grown on V-8 juice agar at 24 C for 3, 7, and 14 days, respectively. Pieces of agar culture (10 × 10 mm) from each isolate of the same group of microorganisms were combined in 100 ml of sterile distilled water in a 250-ml flask. For fungi and actinomycetes, one loopful (~15 mg) of Tween-20 wetting agent was added to each flask. Flasks were shaken in a shaker at 240 excursions per minute for 30 min. The method of Chuang and Ko (3) was used for counting bacteria and actinomycetes. Three milliliters of the microbial suspension was mixed with 30 g of autoclaved soil in a jar. For infestation with a mixture of microorganisms, 1 ml each of fungal, bacterial, and actinomycete suspensions was added. These microbial suspensions were also used to adjust the populations of bacteria and actinomycetes in conducive soil to the same levels as those in suppressive soil.

Twenty grams of suppressive soil mixed with 20 ml of distilled water was adjusted from pH 6.8 to 5.4 with 4 N HCl. Excess water was drained and soil was air-dried in a petri plate overnight at 24 C before being used to make soil blocks for germination tests. Conducive soil was adjusted from pH 5.4 to 6.8 with 1 N KOH, and otherwise treated similarly.

Amendment of soil with selective inhibitors. Ten grams of suppressive or conducive soil was thoroughly mixed with 5 ml of distilled water containing 100 mg of streptomycin sulfate, 5 mg of rose bengal, 100 mg of benomyl (50% wettable powder), or 50 mg of PCNB (75% wettable powder), and used to prepare soil blocks. To determine the effectiveness of selective inhibitors in soil, two sterile absorbent filter paper disks (12.7 mm, Schleicher & Schuell Inc., Keene, NH 03431) placed at the center between two sterile polycarbonate membranes (0.22 μm, 90 mm diameter) were laid on the soil block (50 × 26 × 3 mm) on a glass slide in a plastic petri plate (150 × 25 mm), and covered with another soil block. The two soil blocks were pressed gently to ensure proper diffusion of soil solution to the absorbent disks through the membranes which prevented the disks from becoming contaminated by microorganisms during the experiment. After incubation for 6 hr at 24 C, the absorbent disks were recovered aseptically and each was placed in the center of an agar plate of selective medium mixed with 1 ml of soil suspension at the dilution of 10^{-2} for fungi, 10^{-3} for actinomycetes, or 10^{-4} for bacteria. The width of each inhibition zone was measured after 1 wk for fungi and bacteria and after 2 wk for actinomycetes. To determine the effect of each selective inhibitor on germination of *P. splendens* in aqueous solution, one

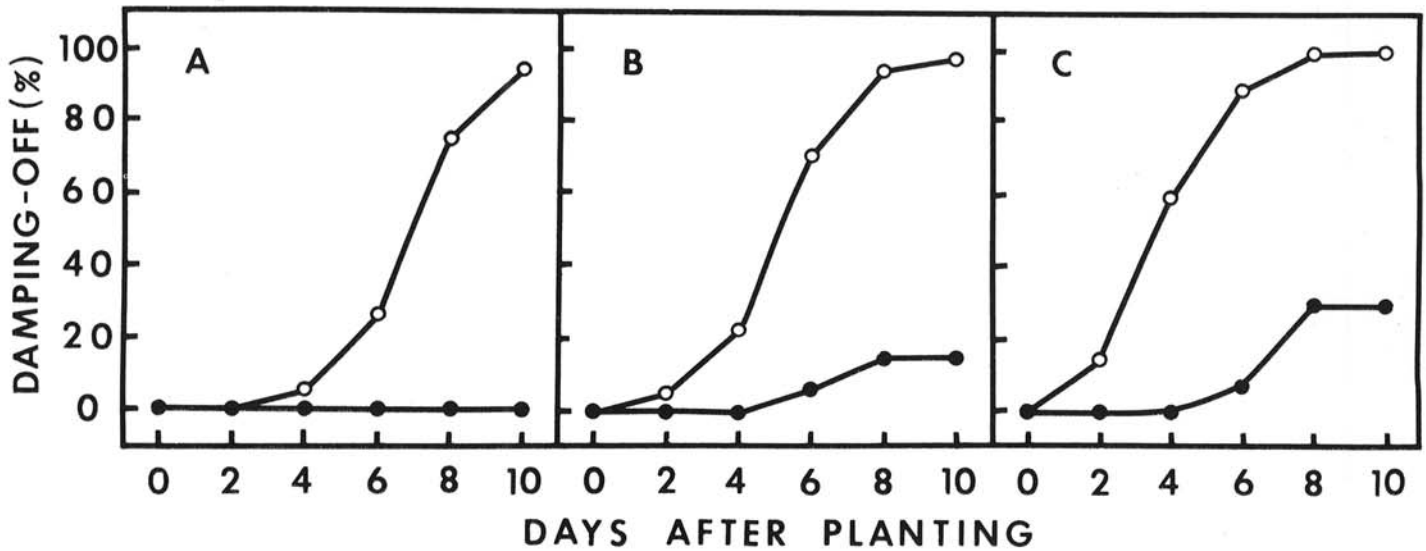

Fig. 1. Damping-off of cucumber seedlings in suppressive (•——•) and conducive (o——o) soils containing: **A,** one sporangium; **B,** three sporangia; and **C,** nine sporangia of *Pythium splendens* per gram of soil.

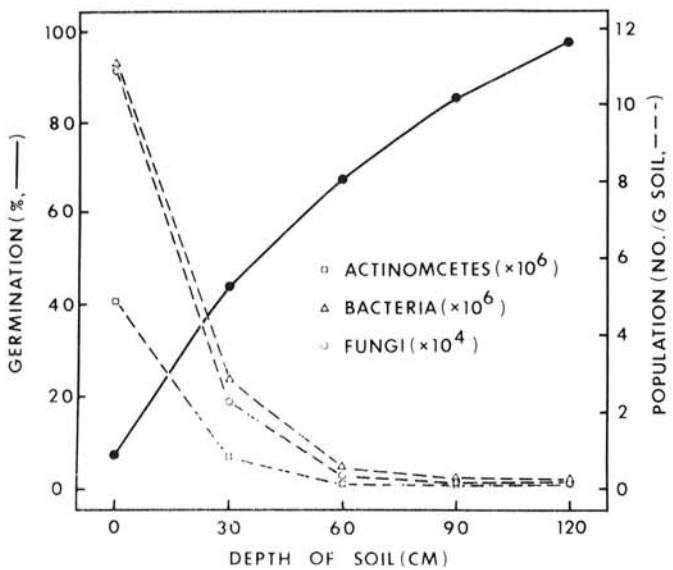

Fig. 2. Germination of sporangia of *Pythium splendens* amended with cucumber root extract and populations of microorganisms in a South Kohala soil at different depths.

drop (~50 μl) of mixture of sporangia and cucumber root extract was added to 2 ml of inhibitor solution at different concentrations in a 6-cm-diameter petri plate. Percent germination was determined after 6 hr at 24 C.

Detection of toxic substances in suppressive soil. For isolation of inhibitors, 50 g of soil was soaked in 50 ml of distilled water in a 150-ml beaker at 24 C for 3 days (15). The resulting soil extract was removed with a pipette and sterilized by passage through polycarbonate membrane (0.22 μm, 47 mm diameter), or used without sterilization. One drop of *P. splendens* sporangial suspension (~5,000 sporangia) was pipetted into 2 ml of soil extract with or without amendment with 20 μl of cucumber root extract in a 6-cm-diameter petri plate. Percent germination was determined based on 100 spores counted after 6 hr. To detect the presence of volatile inhibitors, pieces of double-layer cellophane (30 × 15 mm) were placed on the inside surface of a sterile lid of a petri dish base (100 × 20 mm) which contained 50 g of moistened suppressive soil (12). After 12 hr preincubation at 24 C, *P. splendens* sporangia in cucumber root extract were spread on the cellophane and incubated for 6 hr.

Analysis of data. Three replicates were used for each treatment and all experiments were repeated at least once. Student's *t*-test was used where comparisons were made between two treatments, and Duncan's multiple range test was used when more than two treatments were compared.

TABLE 1. Damping-off of seedlings in suppressive and conducive soils infested with pathogens

Pathogen	Host	Inoculum density (no./g soil)	Seedlings killed (%)[y] Conducive soil	Suppressive soil
Sporangia:				
Pythium splendens	Cucumber	3	98 a[z]	15 b
		9	100 a	30 b
Zoospores:				
P. aphanidermatum	Cucumber	10	94 a	37 b
		100	97 a	73 b
Phytophthora palmivora	Papaya	1,000	13 a	20 a
		10,000	47 a	53 a
P. capsici	Tomato	3,000	46 a	67 a
		30,000	96 a	100 a

[y] Data were from one of two experiments with similar results. Data were recorded 10 days after planting for cucumber, 2 wk for tomato, and 1 mo for papaya.

[z] Data followed by the same letter for each pathogen at each inoculum density were not significantly different ($P = 0.05$) based on Student's *t*-test.

RESULTS

Characteristics of suppression. When compared with conducive soil at the same inoculum density, suppressive soil was suppressive to damping-off of cucumber seedlings caused by *Pythium splendens* and *P. aphanidermatum*, but was not suppressive to damping-off of papaya seedlings caused by *Phytophthora palmivora* or tomato seedlings caused by *P. capsici* (Table 1). Damping-off caused in cucumber seedlings by *P. splendens* was greatly reduced in suppressive soil compared with that in conducive soil (Fig. 1). For example, at the concentration of three sporangia of *P. splendens* per gram of soil, damping-off of cucumber seedlings was 15% in suppressive soil and 98% in conducive soil 10 days after planting.

Germination and growth of microorganisms on suppressive and conducive soil. *P. splendens* sporangia amended with cucumber root extract germinated 15% on suppressive soil and 94% on conducive soil. Germ tubes produced on suppressive soil were shorter and thinner than those on conducive soil after 6 hr. The

TABLE 2. Germination of different fungal spores on soils suppressive and conducive to damping-off of cucumbers caused by *Pythium splendens*

Fungal species	Type of spores	Nutrients added[y]	Germination (%)	
			Conducive soil	Suppressive soil
Pythium aphanidermatum	Zoospores	CRE	86 a[z]	61 b
Pythium ultimum	Sporangia	CRE	77 a	12 b
Phytophthora palmivora	Zoospores	CRE	74 a	71 a
Phytophthora capsici	Zoospores	CRE	95 a	94 a
Mucor hiemalis	Sporangiospores	V-8	76 a	18 b
Mucor ramannianus	Sporangiospores	V-8	88 a	58 b
Neurospora tetrasperma	Ascospores	None	84 a	80 a
Calonectria crotalariae	Ascospores	None	95 a	93 a
	Conidia	None	88 a	89 a
Alternaria alternata	Conidia	V-8	99 a	94 a
Fusarium oxysporum f. sp. *cubense*	Macroconidia	V-8	95 a	4 b
Fusarium oxysporum f. sp. *lycopersici*	Macroconidia	V-8	100 a	48 b

[y] CRE, cucumber root extract; V-8, 25% V-8 juice.
[z] Data followed by the same letter for each fungus are not significantly different ($P = 0.05$) based on Student's t-test.

TABLE 3. Growth of microorganisms on soils suppressive and conducive to damping-off caused in cucumbers by *Pythium splendens*

Microorganism	Relative growth	
	Conducive soil	Suppressive soil
Fungi:		
Rhizoctonia solani	+[a]	+
Sclerotium rolfsii	+	+++
Bacteria:		
Agrobacterium radiobacter	+	++
Pseudomonas solanacearum	+	+
Xanthomonas campestris	+	++
Actinomycetes:		
Nocardia erythropolis	+	+
Streptomyces alboniger	−	++
S. scabies	−	+

[a] Relative growth was based on microscopic observation of microbial cell mass at 6 days for fungi and 24 hr for bacteria and actinomycetes. Symbols: −, no growth; +, slight growth; ++, moderate growth; +++, ample growth.

magnitude of germination inhibition of *P. splendens* by suppressive soil decreased with increasing depth of soil which was associated with decreasing populations of microorganisms (Fig. 2). Germination inhibition and microbial populations of suppressive soil also decreased during storage in the laboratory. After storage for 6 mo, germination of sporangia of *P. splendens* amended with root extract increased from 14 to 61%, and the total microbial population decreased from about 25×10^6 to 8×10^6 per gram of soil.

The suppressive soil was also strongly inhibitory to spore germination of *P. ultimum, M. hiemalis*, and *F. oxysporum* f. sp. *cubense*, and moderately inhibitory to *P. aphanidermatum, M. ramannianus*, and *F. oxysporum* f. sp. *lycopersici*, but was not inhibitory to *Phytophthora palmivora, P. capsici, N. tetrasperma, C. crotalariae*, and *A. alternata* (Table 2). Mycelial growth of *R. solani* on suppressive soil was about the same as that on conducive soil (Table 3). However, *S. rolfsii* grew faster and denser on suppressive soil than on conducive soil. Suppressive soil also stimulated growth of *X. campestris, A. radiobacter, S. alboniger*, and *S. scabies*, but multiplication of *Pseudomonas solanacearum* and *N. erythropolis* on suppressive and conducive soils was about the same.

Restoration of inhibition of sporangial germination to sterilized suppressive soil. Autoclaving, steam treatment, or γ-irradiation of suppressive soil completely nullified its inhibition of germination by sporangia of *Pythium splendens*. Percentage germination increased from the original 18% to 93–97% after treatment. The same treatment did not affect sporangial germination of *P. splendens* on conducive soil. Inhibition was restored to autoclaved suppressive soil after infestation for 7 and 14 days with either suppressive soil suspension or conducive soil suspension (Fig. 3).

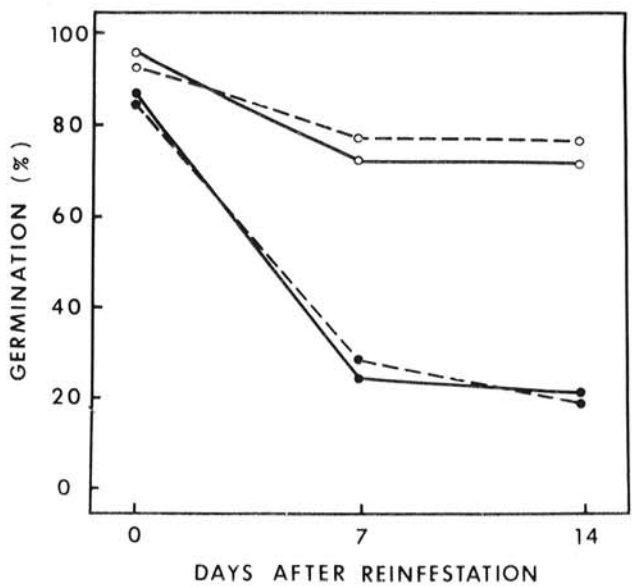

Fig. 3. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on autoclaved suppressive (•) and conducive (o) soils reinfested with suppressive (——) or conducive (– – –) soil suspension.

Both suppressive and conducive soil suspensions failed to change autoclaved conducive soil to become inhibitory to germination, although germination of *P. splendens* sporangia was slightly lower at 7 and 14 days than at day 0. In γ-irradiated suppressive soil infested with either suppressive or conducive soil suspension, inhibition was restored partially after 7 days and completely after 14 days. Neither of the two soil suspensions was able to convert irradiated conducive soils into inhibitory soils 14 days after infestation. Actinomycetes, bacteria, and fungi in separate groups or together were all capable of restoring germination inhibition to autoclaved suppressive soil (Fig. 4). None of the treatments was able to make autoclaved conducive soil inhibitory.

Transfer of germination inhibition from suppressive soil to conducive soil. Conducive soil was mixed with 10, 25, 50, or 75% of suppressive soil and sporangial germination of *P. splendens* amended with root extract was tested in these soils at 0, 14, and 28 days. Germination of sporangia of *P. splendens* on conducive soils decreased with increasing amount of suppressive soil added (Fig. 5). During the incubation periods, germination inhibition in conducive soil mixed with suppressive soil decreased. Similar results were obtained when subsoil collected at the depth of 120 cm in the suppressive field was mixed with various amounts of suppressive soil. Sporangial germination of *P. splendens* on subsoil decreased with increasing amounts of suppressive soil, and germination increased after incubation for 14 and 28 days.

Amendment of suppressive soil with selective microbial inhibitors. Amendment of suppressive soil with selective microbial inhibitors partially nullified its inhibition of sporangial germination. Germination of the sporangia of *P. splendens* amended with root extract was increased from 19% to 57, 47, and 40% by amendment with 333 ppm rose bengal, 6,667 ppm streptomycin, and 3,333 ppm benomyl, respectively (Table 4). PCNB at 2,500 ppm was not effective in reducing suppressiveness of suppressive soil. The inhibitors did not reduce sporangial germination of *P. splendens* in conducive soil at the concentrations used in the test, but did inhibit germination of sporangia at higher concentrations. The values of ED_{50} for inhibition of germination of *P. splendens* in solution for rose bengal, streptomycin, and benomyl were about 40, 150, and 1,000 ppm, respectively. The solution obtained from suppressive soil amended with rose bengal or streptomycin was inhibitory to bacteria and actinomycetes, but not fungi; whereas the solution obtained from benomyl amended soil was inhibitory to fungi, but not bacteria and actinomycetes (Table 5). The solution obtained from PCNB amended soil was not inhibitory to any of the three groups of microorganisms.

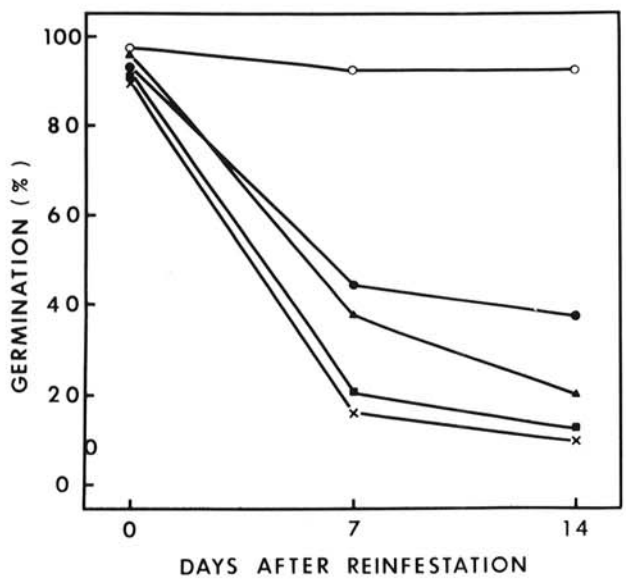

Fig. 4. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on autoclaved suppressive soil reinfested with actinomycetes (•), bacteria (■), fungi (Δ), and the combination of these three groups of microorganisms (×). Autoclaved suppressive soil mixed with sterile distilled water (o) was used as a control.

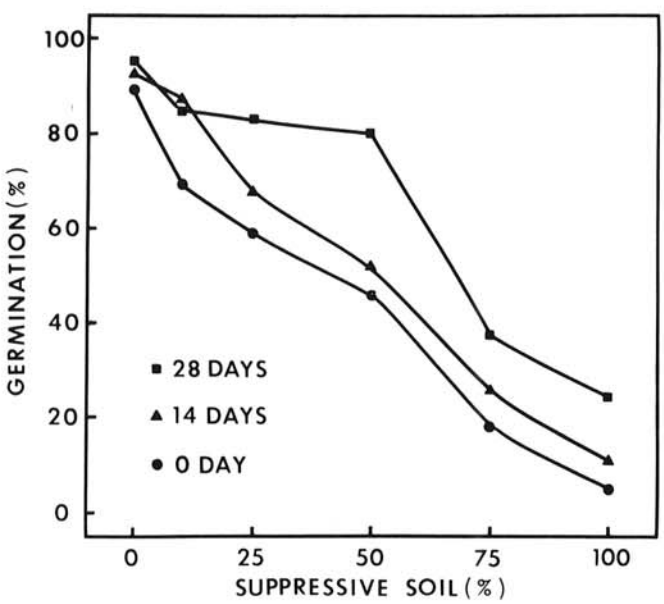

Fig. 5. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on suppressive soil mixed with various amounts of conducive soil.

Detection of inhibitory substances in suppressive soil. When no nutrients were added, sporangia of *P. splendens* failed to germinate in distilled water, and 2–5% germinated in suppressive and conducive soil extracts. Sterilization of soil extracts did not affect spore germination. In the presence of root extract, sporangia of *P. splendens* germinated 95–100% in all treatments, indicating the absence of inhibitory substances in the soil extracts. Sporangia of *P. splendens* amended with root extract germinated completely on cellophane incubated above the surface of suppressive soil and distilled water, indicating the absence of volatile inhibitors.

Adjustment of soil pH and microbial populations. Suppressive soil remained inhibitory to germination of *P. splendens* sporangia when its pH was adjusted from 6.8 to 5.4, and conducive soil remained conducive to germination when its pH was adjusted from 5.4 to 6.8. Before and after pH adjustment, sporangia of *P. splendens* amended with root extract germinated 27–30% on suppressive soils and 93–95% on conducive soils. When the total microbial population in conducive soil was adjusted to about the same as that in suppressive soil by adding mixtures of bacteria and actinomycetes, the soil remained conducive to germination. Sporangia of *P. splendens* amended with root extract germinated about 92% before adjustment and 85% after adjustment.

DISCUSSION

There was a correlation between inhibition of germination of pathogens and suppressiveness to the diseases caused by those pathogens in the soils used in this study. The *Pythium*-suppressive soil was suppressive to damping-off of cucumber caused by *P. splendens* and *P. aphanidermatum*, but it was not suppressive to damping-off of papaya and tomato caused by *P. palmivora* and *P. capsici*, respectively.

The effect of the *Pythium*-suppressive soil from South Kohala on various microorganisms tested ranged from inhibitory to stimulatory when compared with that of conducive soil from Hilo. In addition to the three species of *Pythium* tested, the *Pythium*-suppressive soil was also inhibitory to two species of *Mucor* and

TABLE 4. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on suppressive and conducive soils amended with different microbial inhibitor

Treatment	Concentration (μg/g)	Germination (%)	
		Suppressive soil	Conducive soil
Rose bengal	333	57 a[z]	95 a
Streptomycin	6,667	47 ab	91 a
Benomyl	3,333	40 b	87 a
PCNB	2,500	24 c	92 a
Control		19 c	91 a

[z] Data followed by the same letter in each column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Effect of microbial inhibitors diffused from amended suppressive soil on growth of soil microorganisms in dilution plates

Inhibitor	Concentration (μg/g)	Inhibition zone (mm)[a]		
		Fungi	Bacteria	Actinomycetes
Rose bengal	333	0	8	15
Streptomycin	6,667	0	10	13
Benomyl	3,333	18	0	0
PCNB	2,500	0	0	0
Control		0	0	0

[a] Width of inhibition zone around the filter paper disk. Data were from one of two experiments with similar results.

two formae speciales of *F. oxysporum*. However, the soil had no effect on spore germination of two species of *Phytophthora* and two spore types of *C. crotalariae* which are closely related to *Pythium* and *Fusarium*, respectively, and was even stimulatory to growth of *S. rolfsii*, *X. campestris*, *A. radiobacter*, *S. alboniger*, and *S. scabies*. The suppressive soil also did not affect hyphal growth of *R. solani*.

The inhibition of sporangial germination of *P. splendens* in the South Kohala soil was found to be associated with microbial activity. Sterilization by autoclaving or γ-irradiation, or reduction of microbial populations by steam treatment, completely nullified the inhibitory effect of the suppressive soil. Inhibition was restored to sterilized suppressive soil by reinfestation with soil suspensions or soil microorganisms. Decrease of inhibition in subsoil and stored topsoil was also correlated with decrease in total microbial populations.

The following results suggest that the microorganisms associated with the inhibition of sporangial germination in the South Kohala soil are not specific: Microorganisms from both suppressive soil and conducive soil were able to restore germination inhibition to sterilized suppressive soil. Inhibition of germination was decreased by amendment of suppressive soil with rose bengal, streptomycin, or benomyl. Rose bengal and streptomycin are inhibitory to bacteria and actinomycetes but not to fungi, whereas benomyl is inhibitory to fungi but not to bacteria and actinomycetes. Inhibition of germination was restored to sterilized suppressive soil by reinfestation with bacteria, actinomycetes, or fungi.

Lin and Cook (17) suggested that suppression of lentil root rot caused by *F. roseum* in Washington soil was due to nutrient competition of certain common fast-growing fungi. However, the suppressing effect was not altered by amendment of soil with benomyl. The concentrations (0.25–1.0 ppm) of benomyl they used were probably too low to effectively suppress fungal activity in soil. Alabouvette et al (1) reported that addition of PCNB and rose bengal to soil eliminated the suppressing effect of Chateaurenard soil. This is inconsistent with their claim that saprophytic species of *Fusarium* are responsible for suppressiveness of this soil because neither compound is inhibitory to *Fusarium* species in general (5,20).

Although microorganisms are associated with suppressiveness of South Kohala soil, the microbiological factor alone is insufficient to produce the germination inhibiting effect, because adjustment of total microbial population in conducive soil to the same level as that in suppressive soil did not render it inhibitory to germination. Moreover, the γ-irradiated conducive soil infested with conducive soil suspension remained conducive to germination even though its total microbial population (44×10^6 propagules per gram of soil) was higher than that in natural suppressive soil (25×10^6 propagules per gram of soil) (9). Results of this study suggest that a combination of biotic and abiotic factors is responsible for the suppressing effect of the South Kohala soil.

The nature of the abiotic factor associated with suppressiveness of the South Kohala soil is still unknown. We did not detect any volatile or nonvolatile inhibitors in the suppressive soil. The organic matter content in suppressive soil was similar to that in conducive soil (9). Adjustment of soil pH from 6.8 to 5.4 did not eliminate the germination inhibition factor from the suppressive soil, nor did adjustment from pH 5.4 to 6.8 render the conducive soil inhibitory.

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