Suppression of *Pythium splendens* in a Hawaiian Soil by Calcium and Microorganisms

C. W. Kao and W. H. Ko

Former graduate research assistant and professor, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo 96720. Present address of the first author is Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan.

This research was supported in part by a grant under the USDA Section 406 program.
Journal Series 2977 of the Hawaii Institute of Tropical Agriculture and Human Resources.
Accepted for publication 4 September 1985.

ABSTRACT

Kao, C. W., and Ko, W. H. 1986. Suppression of *Pythium splendens* in a Hawaiian soil by calcium and microorganisms. Phytopathology 76:215-220.

Germination inhibition of sporangia of *Pythium splendens* in seven different soil types was associated with high Ca content and high total microbial population. Conducive soils which were low in Ca content and microbial population became suppressive to *P. splendens* after amendment with 0.6% Ca and mixture of microorganisms, or with Ca and 1% alfalfa meal to increase the population of indigenous microorganisms. Germination of sporangia of *P. splendens* on a suppressive soil from South Kohala, HI, increased with time of storage, and the increase of germination was associated with the decrease of total microbial population. Germination inhibition was restored to suppressive soil stored for 1 and 1.5 yr, by adjusting the moisture content of soils from −110 to −7 bars to increase the microbial population. Suppressiveness also was restored to soil stored for 3 yr by adding fungi, bacteria, actinomycetes, or their combination. Conducive soils which were high in Ca and low in microbial population were converted to suppressive soil by adding alfalfa meal to increase the microbial population. These results suggest that a combination of high Ca and high microbial population is the cause of suppression in the soil from South Kohala. The inhibition characteristics of the suppressive soil created by amending the conducive soil with Ca and alfalfa meal were similar to those of naturally suppressive soil from South Kohala. Naturally and induced suppressive soils were inhibitory or stimulatory to the same species of microorganisms and the inhibitory effect of both soils was associated with microbial activity. Inhibition of sporangial germination of *P. splendens* on both soils decreased when sporangia were separated from soil by a polycarbonate membrane. Neither volatile inhibitors nor nonvolatile inhibitors were detected in either soil. Both soils were fungistatic and their inhibitory effect was not transferable. Similarity of inhibition characteristics indicates that the artificially created suppressive soil operates by the same mechanism as the naturally suppressive soil, and supports the hypothesis that a combination of high Ca and high microbial population is responsible for the suppression of *P. splendens* in the suppressive soil from South Kohala.

Additional key words: soilborne pathogen, suppression mechanism.

By using a simple screening method developed by Ko and Ho (14), a pasture soil in South Kohala on the island of Hawaii was found to be highly suppressive to sporangial germination of *Pythium splendens* Braun (9). In addition, the suppressive soil also was inhibitory to two other species of *Pythium*, two species of *Mucor*, and two *formae speciales* of *Fusarium oxysporum* Schlecht. However, the soil had no effect on spore germination or growth of *Calonectria crotalariae* (Loos) Bell & Sobers, two species of *Phytophthora*, or *Rhizoctonia solani* Kühn, and was even stimulatory to growth of *Sclerotium rolfsii* Saccardo, *Xanthomonas campestris* (Pammel) Dowson, *Agrobacterium radiobacter* (Beijerinck & van Delden) Conn, *Streptomyces alboniger* Porter et al, and *S. scabies* (Thaxter) Waksman & Henrici.

Inhibition of sporangial germination of *P. splendens* on the suppressive soil was associated with microbial activity. Sterilization completely nullified the inhibitory effect of the suppressive soil and inhibition was restored to sterilized suppressive soil by reinfestation with microorganisms. Microbial inhibitors also decreased the inhibitory effect of the suppressive soil. Results from the previous study (9) also suggest that inhibition of *P. splendens* on the soil from South Kohala was associated with high population of microorganisms in general. Decreased inhibition on subsoil and stored topsoil collected from South Kohala was correlated with a decrease in total microbial populations. Moreover, reinfestation with only bacteria, actinomycetes, or fungi also restored the inhibitory effect to the sterilized suppressive soil (9).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Although high microbial population is associated with suppression in the soil from South Kohala, the microbiological factor alone is insufficient to produce the inhibitory effect because adjusting the total microbial population in conducive soil to the same level as that in suppressive soil did not render it inhibitory. This suggested that a combination of an unknown abiotic factor and high microbial population is responsible for the suppressive effect of the soil from South Kohala (9).

The purpose of this study was to identify the abiotic factor which is essential for suppression of *P. splendens* in the soil from South Kohala.

MATERIALS AND METHODS

Soils. Soil suppressive to *Pythium* was collected from a pasture in South Kohala. It was a very fine sandy loam of the Ustollic Eutrandepts subgroup. The soil conducive to *Pythium* collected from the former Hawaii Community College farm in Hilo was a silty clay loam of the Typic Hydrandepts subgroup (9). Other soils used in this study included: a Lualualei clay soil of the Typic Chromusterts subgroup collected from a papaya orchard on the island of Oahu; a Paaloa silty clay soil of the Humoxic Tropohumults subgroup collected from a pineapple field on the island of Oahu; a Waimea very fine sandy loam of the Typic Eutrandepts subgroup collected from a pasture on the island of Hawaii; and a subsoil collected at 60–90 cm depths at South Kohala, HI. Top soils were collected from 0- to 10-cm depths after the surface litter was cleared, sieved through a 2-mm-mesh screen to remove large organic matter, adjusted to about −7 bars moisture, and stored in closed glass jars or polyethylene bags for more than 1 mo before use to exhaust the available nutrients. Air-dried soil with a moisture content about −110 bars was stored for longer periods in a covered iron can or polyethylene bag. Unless otherwise specified, the very fine sandy loam from South Kohala was used as the suppressive soil and the silty clay loam from Hilo was used as the conducive soil.

Microorganisms. The method of Ko and Ho (14) was used to obtain sporangia of *P. splendens* (isolate 106F) and *Pythium ultimum* Trow (isolate 793). Zoospores of *Pythium aphanidermatum* (Edson) Fitzpatrick (isolate 606) were obtained according to the method of Rahimian and Banihashemi (17). Zoospores of *Phytophthora palmivora* (Butler) Butler (isolate 110F) and *Phytophthora capsici* Leonian (isolate P287) were produced by the method described previously (9). Ascospores of *Calonectria crotalariae* (isolate 117F) were obtained according to the method of Hwang and Ko (6). 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 *Fusarium oxysporum* (Schlecht.) f. sp. *lycopersici* (Saccardo) Snyder & Hansen (isolate 119F) were produced on 10% V-8 juice agar under light at 24 C for 7 days. *Sclerotium rolfsii* (isolate 120F) sclerotia were obtained by growing the fungus on potato-dextrose agar under light (cool-white fluorescent, 2,000 lux) at 24 C for 14 days. Mycelial disks of *Rhizoctonia solani* (isolate R434) were obtained according to the method described by Ko and Ho (14). *Xanthomonas campestris* (isolate A249-1), *Pseudomonas solanacearum* (Smith) Smith (isolate AG16), *Nocardia erythropodis* (Gray and Thronton) Waksman and Henrici (isolate 36A), *Streptomyces alboniger* (isolate 23A) and *Streptomyces scabies* (isolate 43A) were grown on 10% V-8 juice agar at 24 C. A loopful of cells or spores from the 2-day-old culture of actinomycetes was suspended in 5 ml of sterile distilled water in a test tube. A small amount of Tween-20 (approximately 0.1 mg) on a needle tip was added to the test tube containing actinomycete spores as a wetting agent. Concentrations of spores were determined by counting the number of spores in 1 μl with a Pipetman digital microliter pipet (P-20D; West Coast Scientific, Inc., Oakland, CA) (12). *Pythium ultimum, P. aphanidermatum,* and *Phytophthora capsici* were obtained from M. Aragaki, and *F. oxysporum* f. sp. *lycopersici* was obtained from W. Nishijima. Bacteria and actinomycetes were obtained from A. M. Alvarez and J. L. Lockwood, respectively.

Germination of fungal spores on soils. Since ascospores of *Calonectria crotalariae* are not sensitive to general soil fungistasis (6), they were added to the soil surface without nutrient amendment. Sporangia of *P. splendens* and other fungal spores are sensitive to general soil fungistasis; therefore, cucumber root extract or V-8 juice were added to overcome that effect. Cucumber root extract was prepared according to the method of Ko and Ho (14). Sporangial or zoospore suspensions of *Pythium* or *Phytophthora* were mixed with equal volumes of cucumber root extract before being added to the soil surface. Spore suspensions of other fungi were mixed with equal volumes of 50% filtered V-8 juice before being placed on the soil. Soil blocks (50 × 26 × 3 mm) with a smooth surface were made on glass slides with a bent spatula as described by Ko and Ho (14). Two drops of spore suspension were added to the surface of a soil block on the glass slide. The slides were then placed in a moist chamber and incubated at 24 C for 6 hr. After incubation, spores were stained with rose bengal (1% rose bengal, 5% phenol, and 0.01% $CaCl_2$ in distilled water). Germination of sporangia of *P. splendens* and *P. ultimum* and conidia of *A. alternata* was counted directly on the soil surface by using a Zeiss Universal Microscope equipped with a model II C vertical illuminator at ×200 (14). Other fungal spores were scraped from the soil surface along with soil particles, placed in a drop of destaining solution (5 M NaOH plus 0.5 M NaCl) on a glass slide, covered with a cover glass, and observed under a compound microscope at ×600 (13). Three replicates were used for each treatment and all the experiments were repeated at least once.

Growth of microorganisms on soils. Mycelia of *R. solani* on a 6-mm-diameter disk of polycarbonate membrane (0.2 μm; Nuclepore Corporation, Pleasanton, CA), or one sclerotium of *S. rolfsii* was placed at one end of a soil block (130 × 40 × 17 mm) in a plastic petri plate (150 × 25 mm). Growth of mycelia on the soil surface was measured every 2 days for 10 days under a stereomicroscope at ×50 (14).

For measuring the growth of bacteria and actinomycetes, the 50 × 30 × 3-mm soil block was made in a 90-mm-diameter petri plate. Five μl of bacterial or actinomycete suspension was pipetted onto a thin water agar sheet (15 × 10 × 1 mm) placed on a sterile polycarbonate membrane (20 × 20 mm) overlaying a soil block. After incubation at 24 C for 24 hr, the water agar sheet was transferred to a glass slide. Microorganisms were stained with cotton blue (100 mg cotton blue in 100 ml of 85% lactic acid), covered with a glass coverslip, and observed under a compound microscope at ×600 or ×1,000. Three replicates were used for each treatment and all experiments were repeated at least once.

Determination of microbial population in soils. Soil suspensions were prepared by blending 10 g of soil with 90 ml of sterile distilled water in an Omni mixer at 4,500 rpm for 2 min and diluted to 10^{-3}, 10^{-4}, and 10^{-5} for fungi; 10^{-4}, 10^{-5} and 10^{-6} for actinomycetes; and 10^{-5}, 10^{-6}, and 10^{-7} for bacteria before plating on selective media. Surfactant-PDA (19), alkaline water agar (5), and PCNB-soil extract agar (4) were used for determining populations of fungi, actinomycetes, and bacteria, respectively. Three plates were used for each dilution and the experiments were repeated at least once.

Determination of calcium content in soils. The method described by Jackson (7) was used to extract exchangeable cations from soil. Calcium content in the solution was determined on a Perkin-Elmer model 603 atomic absorption spectrophotometer.

Soil amendments. To increase the microbial population, a suspension of suppressive soil was plated on selective media and 10 colonies each of bacteria, actinomycetes, and fungi with different morphological appearances were isolated and grown on V-8 juice agar at 24 C for 3, 7, and 14 days, respectively. Bacterial cells or actinomycete spores from isolates of the same group were scraped from the culture surfaces with a bent spatula and suspended in 100 ml of distilled water. Fungal spore suspensions were obtained by combining the washings of culture plates atomized with 10 ml of distilled water. One loopful (approximately 15 mg) of Tween-20 was added to each of the fungal or actinomycete suspensions as a wetting agent, and mixed in an Omni mixer at 4,500 rpm for 30 sec. Thirty grams of soil in a jar was mixed with 3 ml of the microbial suspension. Amendment with mixture of microorganisms consisted of 1 ml each of the fungal, bacterial, and actinomycete suspensions (9). Seven days after amendment with a mixture of microorganisms, the populations of bacteria, actinomycetes, and fungi were 9.1×10^7, 1.9×10^7, and 1.6×10^6 colony-forming units (cfu) per gram of soil, respectively, and the total microbial population was 11.2×10^7 cfu per gram of soil. Fourteen days later, the populations of bacteria, actinomycetes, and fungi were 4.9×10^7, 1.7×10^7, and 9×10^5 cfu per gram of soil, respectively, and the total microbial population was 6.6×10^7 cfu per gram of soil. To increase the population of indigenous microorganisms, soil was mixed thoroughly with 1% alfalfa meal unless otherwise stated. After 7 days, the microbial populations in 1 g of soil were 5.0×10^7, 0.9×10^6, and 7.6×10^5 cfu for bacteria, actinomycetes, and fungi, respectively, and 5.2×10^7 cfu for the total microorganisms. After 14 days, the microbial populations in 1 g of soil were 19×10^7, 7.0×10^6, and 7.9×10^5 cfu for bacteria, actinomycetes, and fungi, respectively, and 19.8×10^7 cfu for the total microorganisms.

To increase the calcium content in soil, $CaCO_3$ was used in most of the experiments; however, other calcium salts such as $Ca(OH)_2$ and $CaSO_4 \cdot 2H_2O$ also were used for comparison. Since the suppressive soil contained about 6 mg Ca/g soil more calcium than the conducive soil, this amount was used for calcium amendments from different sources.

Soil treatment. Methods previously described to characterize suppressive soil (9) were used to characterize the induced suppressive soil prepared by amending conducive soil with 0.6% Ca in the form of $CaCO_3$ and 1% alfalfa meal followed by incubation at 24 C for 14 days. Natural suppressive soil and conducive soil were used as controls. Thirty grams of soil in a 120-ml jar capped with aluminum foil was autoclaved for 15 min at 121 C, exposed to gamma irradiation at 2.5 Mrad, or steamed for 30 min at 97–98 C. To reinfest the soil, 3 ml of conducive soil suspension at 10^{-1} dilution was mixed with sterilized soil in a jar. Sterile distilled water mixed with sterilized soil at the same ratio was used as a control. Soils were assayed for germination of sporangia of *P. splendens* after incubation at 24 C for 7, 14, and 28 days. For treatment of soil

with selective microbial inhibitors, 10 g of soil was mixed thoroughly with 5 ml of distilled water containing 100 mg of streptomycin sulfate, 1 mg of rose bengal, or 50 mg of benomyl (50% wettable powder), and used to prepare soil blocks. To determine the effect of nutrient concentration on inhibition of sporangial germination, sporangia of *P. splendens* were suspended in 0, 1.25, 2.5, 5, 10, and 20% cucumber root extract before being added to the smooth surface of soil blocks. To determine the effect of polycarbonate membrane on germination inhibition of soil, sporangia suspended in 5% cucumber root extract were placed on sterile polycarbonate membrane (0.2 μm, 40 × 20 mm) laid on top of the soil block. Viability of sporangia on the soil surface was determined by placing sporangia without cucumber root extract on the smooth surface of soil blocks made in 90-mm-diameter petri plates. After incubation at 24 C for 2, 4, 6, 8, and 10 days, sporangia along with soil particles were scraped off with a scalpel and suspended in 2 ml of distilled water in a 60-mm-diameter petri plate. A drop (approximately 0.05 ml) of 10% cucumber root extract was added to each plate and germination of sporangia was observed after 3 and 6 hr with a compound microscope.

To determine whether toxic substances were produced in the induced suppressive soil, 50 g of amended 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 sterilized by passage through a polycarbonate membrane (0.2 μm, 47-mm diameter). One drop (approximately 0.05 ml) of a suspension of sporangia of *P. splendens* (approximately 5,000 sporangia) in distilled water or in 5% cucumber root extract was added to 2 ml of soil extract in a 60-mm-diameter petri dish. Percent germination was determined after 3 and 6 hr at 24 C. To detect the presence of volatile inhibitors in the induced suppressive soil, pieces of double-layer cellophane (30 × 15 mm) were placed on the inside surface of a sterile lid of a 90-mm-diameter petri plate which contained 50 g of the induced suppressive soil (13). After preincubation at 24 C for 12 hr, sporangia of *P. splendens* in cucumber root extract were spread on the cellophane and germination was counted after 6 hr. Three replicates were used and all experiments were repeated at least once.

RESULTS

Comparison of sporangial germination, calcium content, and microbial population among soils. Sporangia of *P. splendens* amended with cucumber root extract germinated only 15 and 8%, respectively, on the very fine sandy loam from South Kohala and on the Lualualei clay soil (Table 1). Both soils were high in calcium and microbial populations. More than 90% of the sporangia germinated on the silty clay soil from Hilo and the Paaloa silty clay soil, both of which were low in calcium and microbial population.

TABLE 1. Germination of sporangia of *Pythium splendens* on soils relative to the calcium and total microbial population in these soils[y]

Soil	Germination %	Ca content (meq/100 g soil)	Microbial population (cfu × 10^6/g soil)
S. Kohala, very fine sandy loam	15 a[z]	31.7 b	25.0 a
Lualualei, clay soil	8 a	41.5 a	33.1 a
Hilo, silty clay loam	93 b	1.3 c	9.3 b
Paaloa, silty clay soil	91 b	1.2 c	5.2 b
Waimea, very fine sandy loam	97 b	32.5 b	8.3 b
S. Kohala subsoil	89 b	30.0 b	0.4 c
S. Kohala stored soil	91 b	33.2 b	4.0 b

[y] Sporangia were suspended in 5% aqueous cucumber root extract before being placed on soil.

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

No germination inhibition was found on Waimea soil, subsoil from South Kohala, and stored soil from South Kohala. Although these soils contained about the same amount of Ca as the top soil from South Kohala and Lualualei clay soil, their microbial populations were all relatively low. These results demonstrate that the inhibitory effect of these soils is associated with a combination of high Ca and high total microbial population.

Amendment of conducive soils low in Ca and microorganisms. Amendment of conducive soil from Hilo with $CaCO_3$ or a mixture of microorganisms only slightly decreased sporangial germination of *P. splendens*. However, when both $CaCO_3$ and the mixture of microorganisms were added to the conducive soil, germination was reduced from 94 to 44% (Fig. 1). When 1% alfalfa meal was added to increase the population of indigenous microorganisms in the conducive soil from Hilo (8), sporangial germination was initially reduced from 97 to 48% after 7-day incubation, but the inhibitory effect declined after 14 and 28 days (Fig. 2). Amendment of conducive soil with both $CaCO_3$ and alfalfa meal rendered it strongly inhibitory to germination of *P. splendens* after 7 days, and the inhibitory effect remained very strong after 28 days. Even after 3 mo, the soil was still inhibitory to germination of sporangia of *P. splendens*. Similar results were obtained when conducive soil from Paaloa was amended with $CaCO_3$, alfalfa meal, or $CaCO_3$ plus alfalfa meal where germination of sporangia of *P. splendens* was decreased from 92 to 58, 50, and 28%, respectively, 14 days after amendment. In a separate experiment where different sources of Ca were used to amend conducive soil from Hilo plus 1% alfalfa meal, sporangia of *P. splendens* germinated 24, 16, and 31% on soils amended with $CaCO_3$, $Ca(OH)_2$, and $CaSO_4$, respectively.

Amendment of conducive soils high in Ca and low in microorganisms. Germination of sporangia of *P. splendens* amended with cucumber root extract on soil from South Kohala increased as the time of storage increased and was associated with the decrease of total microbial population in the soil (Fig. 3). After 12-mo storage, the microbial population decreased from 24.5 × 10^6 to 3.1 × 10^6 cfu/g of soil, and sporangial germination increased from 17 to 91%. When the moisture content of stored soils from South Kohala was adjusted from −110 to −7 bars, germination of sporangia of *P. splendens* decreased with increasing time of incubation (Fig. 4). After incubation for 28 days at 24 C, sporangia germinated only 8 and 3% on soils from South Kohala stored for 1 and 1.5 yr, respectively. The decreased sporangial germination on remoistened soil from South Kohala stored for 1.5 yr was

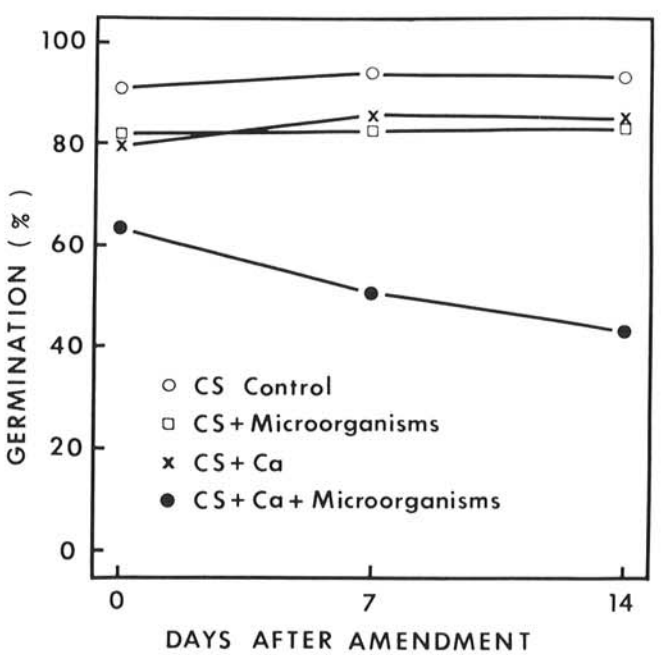

Fig. 1. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on conducive soil (CS) amended with $CaCO_3$ (X), microorganisms (□), or $CaCO_3$ plus microorganisms (●).

associated with an increase in total microbial population (Fig. 5). Suppressiveness was only partially restored by remoistening soil from South Kohala stored for 3 yr (Fig. 4). However, when this soil was amended with fungi, bacteria, actinomycetes, or their combination, germination of *P. splendens* decreased drastically within one day (Fig. 6). Bacteria, and the combination of all three groups of microorganisms, were the most effective treatments to restore suppressiveness.

Subsoil from the South Kohala and Waimea top soil were both high in Ca and low in microbial population. When these soils were amended with 1% alfalfa meal and incubated for 14 days, germination of sporangia of *P. splendens* was decreased from 94 to 21% on South Kohala subsoil and from 95 to 18% on Waimea top soil.

Comparison of induced suppressive soil and naturally suppressive soil for inhibition spectrum. The induced suppressive soil and the naturally suppressive soil were similar in their inhibitory effect on fungal spore germination. The two soils were both highly suppressive to sporangial germination of *Pythium ultimum*, and moderately suppressive to zoospore germination of *P. aphanidermatum* and macroconidial germination of *F. oxysporum* f. sp. *cubense* and *F. oxysporum* f. sp. *lycopersici*, but were not inhibitory to spore germination of *Phytophthora capsici*, *P. palmivora*, *C. crotalariae*, and *A. alternata*.

Growth of microorganisms on induced suppressive soil and naturally suppressive soil also was quite similar. Mycelial growth of *R. solani* on induced or naturally suppressive soils was about the same as that on conducive soil. However, mycelia of *S. rolfsii* grew better on both induced and naturally suppressive soils than on conducive soil, although growth on naturally suppressive soil was slightly faster and denser than on the induced suppressive soil. Both induced suppressive soil and naturally suppressive soil were stimulatory to growth of *Xanthomonas campestris, Streptomyces scabies*, and *S. alboniger*. *Pseudomonas solanacearum* showed

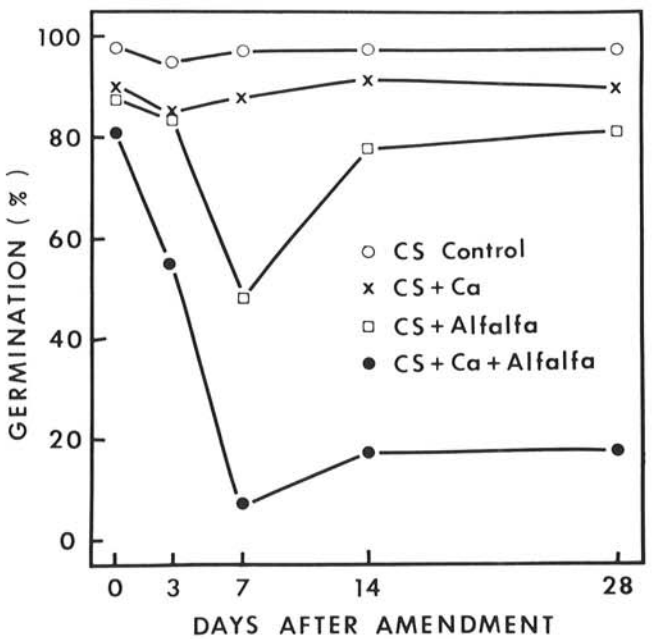

Fig. 2. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on conducive soil (CS) amended with $CaCO_3$ (X), alfalfa meal (□), or $CaCO_3$ plus alfalfa meal (•).

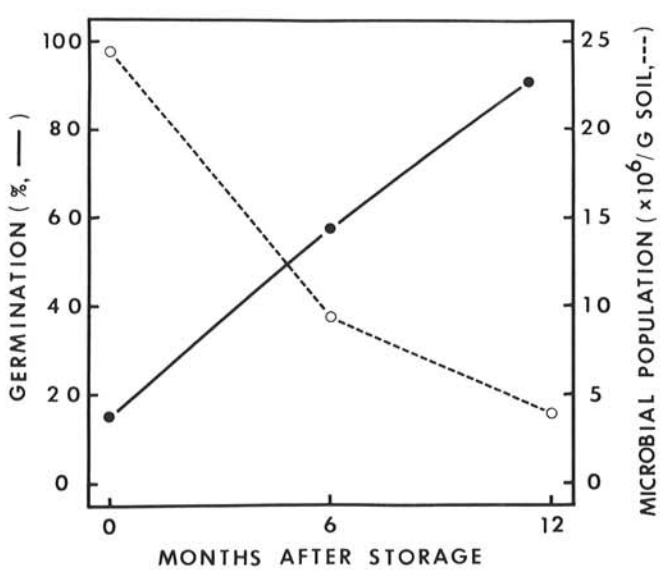

Fig. 3. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on stored soils from South Kohala relative to the total soil microbial population.

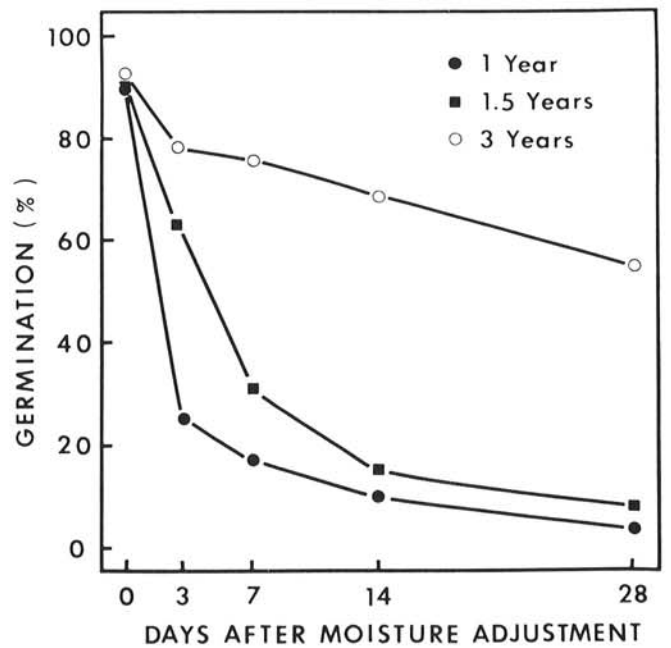

Fig. 4. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on remoistened soil from South Kohala stored at air-dried moisture for 1, 1.5, and 3 yr.

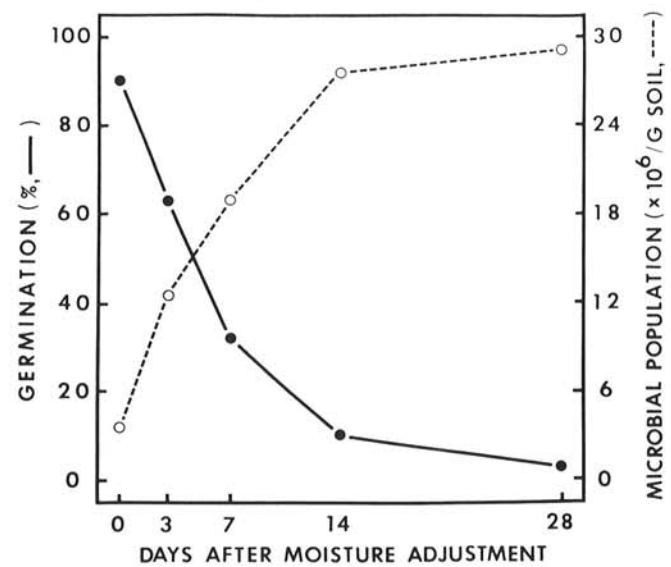

Fig. 5. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on stored soil from South Kohala (1.5 yr) after moisture adjustment relative to the total microbial population.

poor growth and *Nocardia erythropodis* did not grow on these three soils.

Restoration of inhibition to treated soils. Steam treatment, autoclaving or gamma irradiation of the induced suppressive soil or naturally suppressive soil completely nullified the inhibitory effect on sporangial germination of *P. splendens*. Germination increased from 10 to 92–97% after treatments. Inhibition of sporangial germination was restored to steamed induced suppressive soil and naturally suppressive soil but not to steamed conducive soil after infestation with conducive soil suspension for 7 and 14 days (Fig. 7). Similar results were obtained when the conducive soil suspension was used to reinfest gamma-irradiated induced suppressive soil and naturally suppressive soil.

Effect of selective microbial inhibitors on germination inhibition. Amendment of the induced suppressive soil with selective microbial inhibitors partially nullified its inhibitory effect on sporangial germination of *P. splendens*. Germination increased from 23 to 59, 50, and 37% by amendment with 1,667 ppm benomyl, 6,667 ppm streptomycin sulfate, and 67 ppm rose bengal, respectively.

Effect of polycarbonate membrane on germination inhibition of soils. Interpositioning of a polycarbonate membrane between the soil and sporangia reduced the inhibition of germination of *P. splendens* on both the induced suppressive soil and naturally suppressive soil. Germination of sporangia was 65% with membrane and 17% without membrane on the induced suppressive soil; and 68% with membrane and 15% without membrane on the naturally suppressive soil.

Viability of sporangia of *P. splendens* after exposure to soils. Incubation of sporangia of *P. splendens* on induced or naturally suppressive soil for up to 10 days did not affect the ability of sporangia to germinate since more than 90% of these sporangia germinated after being transferred to a nutrient solution for 3 hr. These results suggest that the inhibitory effect of both soils is fungistatic.

Detection of inhibitory substances in the induced suppressive soil. Only 3% of sporangia of *P. splendens* in the unamended extract of induced suppressive soil germinated. Addition of cucumber root extract stimulated sporangia of *P. splendens* to germinate completely. These results indicated that inhibitory substances were absent from the soil extract. Volatile inhibitors also were not detected in the induced suppressive soil because sporangia of *P. splendens* amended with cucumber root extract germinated 98% on cellophane membranes suspended above the surface of the induced suppressive soil in a petri plate.

Transferability of inhibition from induced suppressive soil to conducive soil. Conducive soil was mixed with 25, 50, or 75% of the induced suppressive soil and germination of sporangia of *P. splendens* amended with cucumber root extract were tested in these soils at 0, 7, and 14 days. Germination of sporangia on conducive soils decreased with increasing ratios of induced suppressive soil (Fig. 8). The inhibitory effect did not increase during the incubation period indicating that it was not transferable.

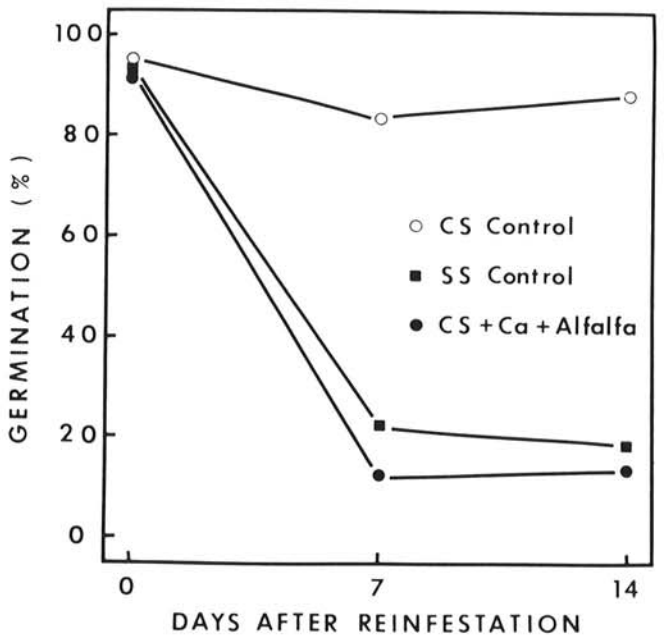

Fig. 7. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on steamed conducive soil (o), steamed suppressive soil (■), or steamed conducive soil amended with $CaCO_3$ and alfalfa meal (•). Germination was determined 0, 7, and 14 days after reinfestation of steamed soils with a conducive soil suspension.

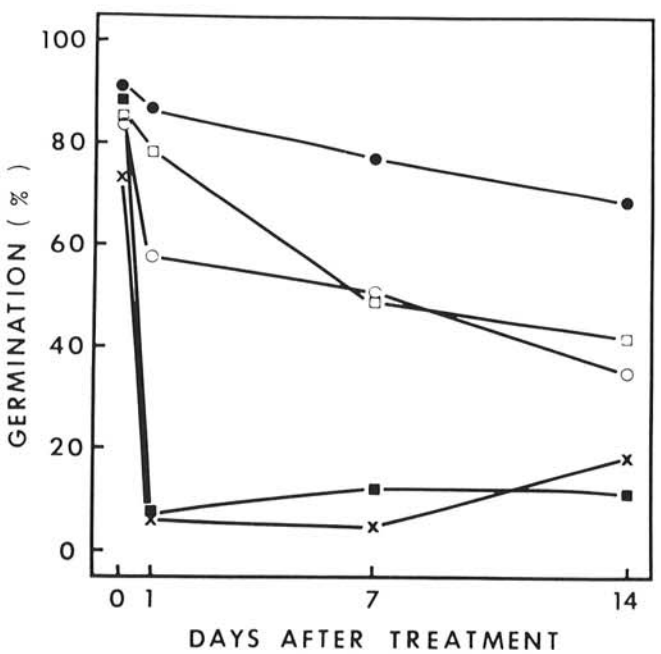

Fig. 6. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on stored soil from South Kohala (3 yr) mixed with actinomycetes (□), bacteria (X), fungi (o), or a combination of actinomycetes, bacteria, and fungi (■). Stored soil mixed with sterile distilled water (•) was used as the control.

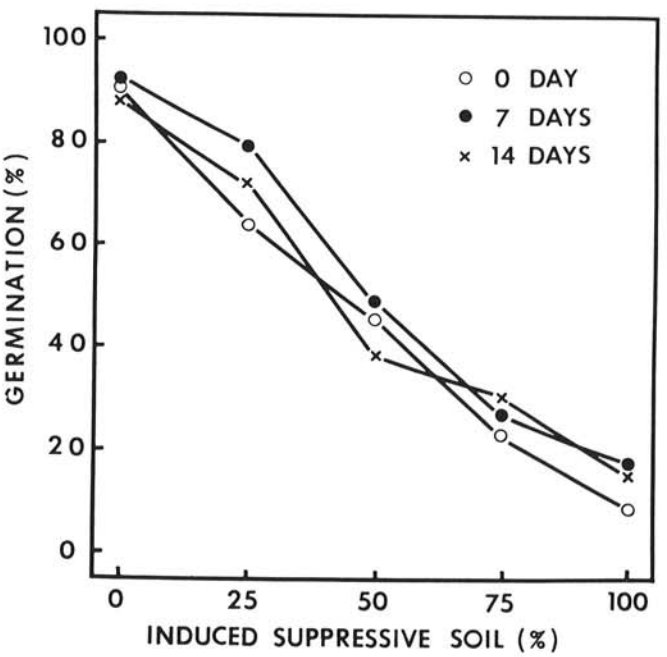

Fig. 8. Germination of sporangia of *Pythium splendens* amended with cucumber root extract on conducive soil mixed with various amounts of the induced suppressive soil (conducive soil + $CaCO_3$ + alfalfa meal).

DISCUSSION

A previous study suggested that a combination of an unknown abiotic factor and high microbial population was responsible for the suppression of *P. splendens* in a soil from South Kohala, HI (9). Results from this study suggest that Ca is the unknown abiotic factor and that a combination of high Ca and high microbial population is the cause of suppression of *P. splendens* in this soil. These include the association of high Ca content and high microbial population with suppression of sporangial germination in seven different types of soil; the ability to transfer conducive soils low in Ca and microbial populations into soil suppressive to *P. splendens* after amendment with Ca and mixtures of microorganisms, or Ca and alfalfa meal to increase the population of indigenous microorganisms; and the conversion of conducive soils which were high in Ca but low in microbial population to suppressive soils by increasing the microbial population.

The inhibition characteristics of the suppressive soil created by amending conducive soil from Hilo with Ca and alfalfa meal were similar to those of the naturally suppressive soil from South Kohala. Characteristics compared include: (i) Association with microbial activity. Sterilization nullified the inhibitory effect of both naturally and induced suppressive soils. However, the inhibitory effect was restored to sterilized soils by reinfestation with soil microorganisms in both cases. (ii) Spectrum of pathogen suppression. Naturally and induced suppressive soils were inhibitory or stimulatory to the same species of microorganisms. (iii) Spectrum of disease suppression. Naturally and induced suppressive soils were suppressive or conducive to the same diseases of plants tested (8). (iv) Response to selective inhibitors. Streptomycin, rose bengal, and benomyl reduced the inhibitory effect of both naturally and induced suppressive soils (9). (v) Response to nutrient amendment. The inhibitory effect of naturally and induced suppressive soils were counteracted by nutrients with similar dosage response curves (10). (vi) Effect of separation by polycarbonate membrane. Inhibition of sporangial germination on both naturally and induced suppressive soils decreased when sporangia were separated from soil by a polycarbonate membrane. (vii) Involvement of inhibitory substances. Neither volatile inhibitors nor nonvolatile inhibitors were detected in either naturally or induced suppressive soil (9). (viii) Mode of inhibition. Both soils were fungistatic. (ix) Transferability. The suppressiveness of either soil was not transferable (9). The similarity in inhibition characteristics suggests that the suppressive soil created artificially operates by the same mechanism operative in the naturally suppressive soil (11). These results, therefore, support the hypothesis that a combination of high Ca and high microbial population is responsible for the suppression of *P. splendens* in soil from South Kohala.

Transfer of the suppressive factor from a suppressive soil to a naturally conducive soil means that the suppressive factor can multiply successfully in the new environment. Therefore, the level of suppression in the conducive soil should increase within a few days of mixing with suppressive soil. The suppressive factor in the soil from South Kohala was considered nontransferable because the inhibitory effect of the conducive soil mixed with suppressive soil was in proportion to the amount of induced or naturally suppressive soil added and did not increase during the 2-wk incubation period (9). The suppressive factors of the potato scab-suppressive soil and Fusarium wilt-suppressive soil were reported to be transferable by Menzies (16) and Alabouvette et al (1), respectively, based on the observations that conducive soils became suppressive after mixing with different proportions of suppressive soils. However, since both reports did not show whether the suppressive effect increased after mixing, the possibility that the effect was entirely that of the original suppressive soil can not be ruled out.

Successful transfer of the suppressive factor from the suppressive soil to a nutrient-amended conducive soil, or to a sterilized or partially sterilized soil will require that the mechanism of suppression of the treated soil induced by mixing with suppressive soil is exactly the same as that operating naturally. Identity of the mechanism of suppression could be determined by a careful comparison of the inhibition characteristics of naturally and induced suppressive soil (11). This has not been done in any of the previous studies of the transferability of suppressive factors (2,3). For example, Scher and Baker (18) found that steamed conducive soil became suppressive to Fusarium wilt of flax after mixing with small amounts of suppressive soil. This was considered to be a successful transfer of the suppressive factor from the suppressive soil to steamed conducive soil. However, the characteristics of the induced suppression were not compared with that of the naturally suppressive soil. Therefore, one can not be sure that the mechanism of suppression induced by mixing small amounts of suppressive soil with steamed conducive soil is the same as that of naturally suppressive soil.

LITERATURE CITED

1. Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of *Fusarium* wilt-suppressive soils and prospects for their utilization in biological control. Pages 165-182 in: Soil-borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, London.
2. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman, San Francisco.
3. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN.
4. Farley, J. D., and Lockwood, J. L. 1969. The suppression of actinomycetes by PCNB in culture media for enumerating soil bacteria. Phytopathology 58:714-715.
5. Ho, W. C., and Ko, W. H. 1980. A simple medium for selective isolation and enumeration of soil actinomycetes. Ann. Phytopathol. Soc. Jpn. 46:634-638.
6. Hwang, S. C., and Ko, W. H. 1974. Germination of *Calonectria crotalariae* conidia and ascospores on soil. Mycologia 66:1053-1055.
7. Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.
8. Kao, C. W. 1985. The role of calcium and microorganisms in suppression of *Pythium splendens* in a pasture soil from South Kohala, Hawaii. Ph.D. thesis. University of Hawaii, Honolulu. 97 pp.
9. Kao, C. W., and Ko, W. H. 1983. Nature of suppression of *Pythium splendens* in a pasture soil in South Kohala, Hawaii. Phytopathology 73:1284-1289.
10. Kao, C. W., and Ko, W. H. 1986. The role of calcium and microorganisms in suppression of cucumber damping-off caused by *Pythium splendens* in a Hawaiian soil. Phytopathology 76:221-224.
11. Ko, W. H. 1985. Natural suppression of soil-borne plant diseases. Plant Prot. Bull. (Taiwan) 27:171-178.
12. Ko, W. H., Chase, L. L., and Kunimoto, R. K. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63:1206-1207.
13. Ko, W. H., and Chow, F. K. 1978. Soil fungistasis: Role of volatile inhibitors in two soils. J. Gen. Microbiol. 104:75-78.
14. Ko, W. H., and Ho, W. C. 1983. Screening soils for suppressiveness to *Rhizoctonia solani* and *Pythium splendens*. Ann. Phytopathol. Soc. Jpn. 49:1-9.
15. Ko, W. H., Hora, F. K., and Herlicska, E. 1974. Isolation and identification of a volatile fungistatic substance from alkaline soil. Phytopathology 64:1398-1400.
16. Menzies, J. D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648-652.
17. Rahimian, M. K., and Banihashemi, Z. 1979. A method for obtaining zoospores of *Pythium aphanidermatum* and their use in determining cucurbit seedling resistance to damping-off. Plant Dis. Rep. 63:658-661.
18. Scher, F. M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.
19. Steiner, G. W., and Watson, R. D. 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology 55:728-730.