Nature of Suppression of *Phytophthora capsici* in a Hawaiian Soil

W. H. Ko and K. A. Nishijima

Professor and research associate, respectively, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo 96720.

This research was supported in part by a grant under the USDA Section 406 Program. We thank R. J. Cook for determination of soil-water potential.

Accepted for publication 9 January 1985.

ABSTRACT

Ko, W. H., and Nishijima, K. A. 1985. Nature of suppression of *Phytophthora capsici* in a Hawaiian soil. Phytopathology 75:683-685.

A forest soil from South Hilo District on the island of Hawaii suppressed germination of sporangia of *Phytophthora capsici* and also damping-off caused in tomato seedlings by the fungus. The soil was also strongly inhibitory to spore germination of *Phytophthora palmivora, Mucor ramannianus, Calonectria crotalariae*, and *Penicillium frequentans*, but it was not inhibitory to spore germination of *Pythium splendens* or *Neurospora tetrasperma*, or to sclerotium germination of *Sclerotium rolfsii*. The suppressive soil was fungitoxic to sporangia of *P. capsici* and the germination inhibition by the soil was not affected by addition of nutrients. Although low pH was inhibitory to *P. capsici*, it alone could not account for the strong inhibitory effect of the suppressive soil. The inhibitory effect was not changed by autoclaving, ignition at 500 C for 16 hr, or H_2O_2 treatment. The inhibitory factor was neither water soluble nor diffusible. The suppression was not affected by amendment of soil with chelating agents. The clay, silt and sand fractions of the suppressive soil were all inhibitory to *P. capsici*. Results suggest that inorganic soil particles are responsible for the inhibition of germination of sporangia of *P. capsici* in the South Hilo soil.

A simple screening method developed for detecting pathogen-suppressive soils has been used to identify soils suppressive to *Pythium splendens* Braun and *Rhizoctonia solani* Kühn in Hawaii (9), and their suppression mechanisms have been investigated (5,14). Since several species of *Phytophthora* are important soilborne plant pathogens (6), the method was employed to search for soils suppressive to *Phytophthora* spp. A forest soil on the island of Hawaii was consequently found to be suppressive to *Phytophthora capsici* Leonian.

The purpose of the studies reported here was to investigate the nature of fungal suppression in this soil. A brief account of this work has been reported (13).

MATERIALS AND METHODS

Soils. Soils suppressive or conducive to *P. capsici* were collected from South Hilo District and South Kohala District, respectively, on the island of Hawaii. Soils were collected from depths ranging from 0 to 10 cm after the surface litter had been cleared, sieved through a 2-mm screen, adjusted to about −7 bars moisture, and stored in closed glass jars at 24 C. Characteristics of both suppressive and conducive soils are presented in Table 1 (16).

To extract inhibitory substances, 25 g of soil were shaken with 25 ml of distilled water in a 250-ml flask (10). After 48 hr, the soil suspension was centrifuged at 12,000 *g* for 20 min. Sporangial germination was tested in 2 ml of the supernatant in a small petri dish (50 × 15 mm). To separate sand, silt, and clay from the soil, one part of the soil was mixed with 10 parts of water (w/w) in a Waring blender for 20 min and the resulting soil suspension was passed through a 44-μm sieve (4,11). Particles remaining on the sieve were washed free of silt and clay with water and used as the sand fraction. The filtrate was centrifuged at 75 *g* for 5 min, and the sediment was used as the silt fraction. The supernatant was further centrifuged at 1,085 *g* for 10 min, and the sediment was used as the clay fraction.

Microorganisms. Sporangia of *P. capsici* (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, 2,000 lux) for 5–7 days at 24 C. The method of Ko and Ho (9) was used to obtain sporangia of *P. splendens* (isolate #106F). Conidia of *Calonectria crotalariae* (Loos) Bell & Sobers (isolate #117F) were obtained according to the method of Hwang and Ko (3). *Neurospora tetrasperma* Shear & Dodge (isolate #38F) ascospores were obtained as described by Ko and Lockwood (12) and used after heat activation at 58 C for 20 min. Conidia of *Alternaria alternata* (Fries) Keissler (isolate #111F) and *Bipolaris maydis* (Nishikado) Shoemaker (isolate #280) were produced by growing the fungi on 10% V-8 juice in darkness at 24 C for 7 days (1). Sporangiospores of *Mucor ramannianus* Moeller (isolate #41F), conidia of *Penicillium frequentans* Westling and macroconidia of *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Saccardo) Snyder & Hansen (isolate #119F) were obtained by growing the fungus on 10% V-8 juice agar under light at 24 C for 7 days. Sclerotia of *Sclerotium rolfsii* Saccardo (isolate #120F) were produced by growing the fungus on potato-dextrose agar under light at 24 C for 14 days. *P. capsici* and *B. maydis* were obtained from M. Aragaki, University of Hawaii, Honolulu, and *F. oxysporum* f. sp. *lycopersici* was obtained from W. Nishijima, University of Hawaii, Hilo.

Germination of fungal propagules on soils. Sporangia of *Phytophthora* usually germinate indirectly by releasing zoospores in the absence of nutrients and directly by producing germ tubes in the presence of nutrients (2). Therefore, sporangia of *P. capsici* were preincubated in 50% V-8 juice for 1 hr to ensure uniform direct germination before being futher incubated on the soil surface for 4 hr at 24 C as described by Ko and Ho (9). The 50% V-8 juice was prepared by mixing V-8 juice with 0.1% $CaCO_3$ and filtering the mixture through Whatman No. 1 filter paper. The filtrate was adjusted to pH 6 with 1 N KOH and diluted with an equal volume of water before use. Ascospores of *N. tetrasperma* and conidia of *C. crotalariae* were added to the soil without nutrient amendment as they are not sensitive to general soil fungistasis (3,12). Sclerotia of *S. rolfsii* were also placed directly on the soil surface. For spores of other fungi, nutrients were added to overcome general soil fungistasis. Sporangia of *P. splendens* were preincubated in 50% V-8 juice for 1 hr before being added to the soil. Spores of other fungi were suspended in 50% V-8 juice and added to the soil immediately. Germination of propagules was counted directly on the soil surface (9). One hundred spores or 10 sclerotia were counted for each replicate. Two replicates were used for each treatment and the experiments were repeated at least once.

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.

Assay of disease severity. About 45 g of soil in a plastic container (75 × 55 × 55 mm) were mixed with 10 ml of sporangial suspension of *P. capsici* and planted with 10 tomato seeds (cultivar N-52). Three spore concentrations and two pots per concentration were used. Concentration of spores was determined with a Pipetman digital microliter pipet (P-20D; West Coast Scientific, Inc., Oakland, CA) (8). Data were recorded after 2 wk. Diseased seedlings were washed with tap water, blotted dry on paper towel and plated on a selective medium (7) to ensure that the disease was caused by *P. capsici*. The experiments were repeated twice.

RESULTS AND DISCUSSION

Characteristics of suppression. Soil suppressive to *P. capsici* was also suppressive to the disease caused by the pathogen (Fig. 1). For instance, at the concentration of 10,000 sporangia of *P. capsici* per gram of soil, damping-off of tomato seedlings was 0% in suppressive soil and 92% in conducive soil. The suppressive soil was also strongly inhibitory to spore germination of *P. palmivora, M. ramannianus, C. crotalariae,* and *P. frequentans*. It was moderately inhibitory to spores of *F. oxysporum* f. sp. *lycopersici, B. maydis,* and *A. alternata*; but the soil was not inhibitory to *P. splendens* and *N. tetrasperma* (Table 2). Germination of sclerotia of *S. rolfsii* was also not inhibited by the suppressive soil.

To determine if inhibition of spore germination by suppressive soil was fungistatic, sporangia of *P. capsici* were incubated on suppressive soil for 24 hr at 24 C, scraped from the soil surface with a spatula, incubated in 2 ml of distilled water or 50% V-8 juice in a test tube for 4 hr at 24 C and then counted in a small petri dish after being stained with rose bengal. After exposure to suppressive soil for 24 hr, only 25 and 30% of sporangia were able to germinate in water and in 50% V-8 juice, respectively. More than 90% of sporangia germinated after exposure to conducive soil for 1 min. Without exposure to soil, germination in water and 50% V-8 juice was 96 and 83%, respectively. Apparently, the suppressive soil is fungitoxic to sporangia of *P. capsici*. Sporangia of *P. capsici* were suspended in different concentrations of V-8 juice for 1 hr before being added to the soil surface, and germination was counted after incubation for 4 hr at 24 C to determine the effect of nutrients on inhibition by suppressive soil. Germination of sporangia coated with 25, 50, and 100% V-8 juice was 10, 10 and 12%, respectively. In another experiment, about 8 g of suppressive soil were moistened with approximately 1 ml of 50% V-8 juice and used to prepare a soil block as previously described (9). Germination of sporangia of *P. capsici* on the nutrient-amended soil was 4% as compared to 1% on the nonamended soil. The results indicate that nutrients have no effect on inhibition of germination by the suppressive soil.

Conducive soil was mixed with 25, 50, or 75% suppressive soil and assayed for germination of sporangia of *P. capsici*. About 75% suppressive soil was required for 50% inhibition of sporangial germination in conducive soil. Soil samples were taken at different depths from the suppressive area and tested for ability to support sporangial germination. Germination on soils collected at depths of 0, 30, 45, 60, and 75 cm was 2, 6, 3, 9, and 10%, respectively, indicating that subsoil is also inhibitory to *P. capsici*.

When the reaction of the suppressive soil was adjusted from the original pH 4.2 to 7.0 with 2 N KOH, sporangial germination increased from 3 to 35%. Adjusting the reaction of conducive soil from the original pH 7.0 to 4.2 with 2 N HCl decreased the germination from 92 to 30%. The results suggest that although low

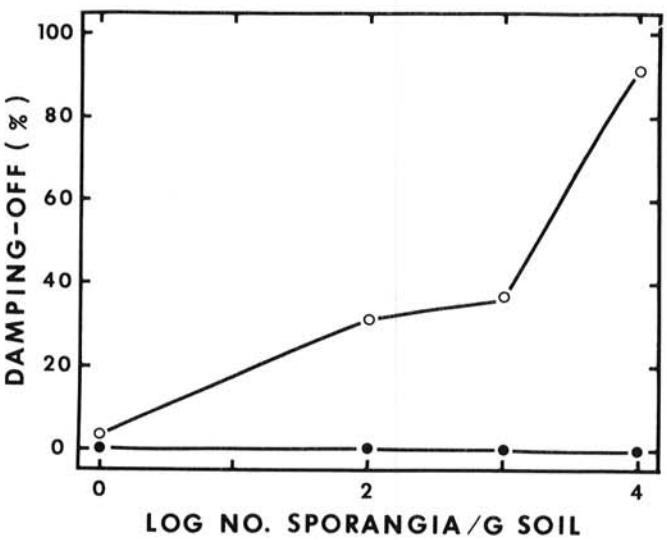

Fig. 1. Damping-off caused in tomato seedlings by *Phytophthora capsici* in suppressive (•——•) and conducive (o——o) soils. Data were from one of three experiments with similar results. Each point was based on 20 seeds planted in two pots. Data were recorded 2 wk after planting.

TABLE 1. Characteristics of two Hawaiian soils, one conducive and the other suppressive to *Phytophthora capsici*

Soil	Classification	Type	pH	Total chemical analysis (%)[z]				
				SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO_2
Suppressive	Typic Hydrandept	Silty clay loam	4.2	13	5	24	28	0.2
Conducive	Typic Eutrandept	Very fine sandy loam	7.0	28	3	21	13	0.3

[z]Data from Soil Survey Investigations Report No. 29 (reference 16).

TABLE 2. Germination of different fungal propagules on two Hawaiian soils, one conducive and one suppressive to *Phytophthora capsici*

Fungal species	Type of propagules	Nutrients added[y]	Germination (%)[z]	
			Suppressive soil	Conducive soil
Phytophthora palmivora	Sporangia	V-8	0 a	83 b
Pythium splendens	Sporangia	V-8	82 a	97 a
Mucor ramannianus	Sporangiospores	V-8	11 a	92 b
Calonectria crotalariae	Conidia	None	12 a	87 b
Fusarium oxysporum f. sp. *lycopersici*	Macroconidia	V-8	62 a	93 b
Penicillium frequentans	Conidia	V-8	13 a	83 b
Bipolaris maydis	Conidia	V-8	46 a	88 b
Alternaria alternata	Conidia	V-8	45 a	89 b
Neurospora tetrasperma	Ascospores	None	91 a	98 a
Sclerotium rolfsii	Sclerotia	None	95 a	95 a

[y]V-8 means 50% V-8 juice.

[z]Germination of propagules was counted directly on the soil surface. One hundred spores or 10 sclerotia were counted for each replicate. Two replicates were used for each treatment and experiments were repeated at least once. Data followed by the same letter for each fungus are not significantly different ($P = 0.05$) based on Student's T-test.

TABLE 3. Effects of soil treatments on germination of sporangia of *Phytophthora capsici*

Treatment[y]	Germination (%)[z]	
	Suppressive soil	Conducive soil
None	0–10	74–91
Autoclaving	0	84
H_2O_2	0	71
Ignition	1	5
Extraction, aqueous extract	97	88
Chelating agents:		
EDTA	7	82
EDDHA	7	80
Membrane separation	92	96
Fractionation:		
Sand	3	80
Silt	2	76
Clay	1	78

[y] EDTA = ethylenediaminetetraacetic acid; EDDHA = ethylenediaminedi-*o*-hydroxyphenylacetic acid. Soil was separated into sand, silt, and clay fractions by sedimentation and sieving.

[z] Germination was counted directly on the soil surface. One hundred spores were counted for each replicate and two replicates were used for each treatment. Data were from one of two experiments with similar results.

pH is inhibitory to *P. capsici*, it alone cannot account for the strong inhibitory effect of the South Hilo soil against this pathogen.

Mechanism of suppression. Sporangial germination of *P. capsici* was completely inhibited on autoclaved suppressive soil (Table 3), which indicates that living organisms were not responsible for the suppression. The inhibitory effect of the suppressive soil was not decreased after treatment with H_2O_2 (15) or ignition at 500 C for 16 hr to remove organic matter. The results suggest that the inhibitory factor in the suppressive soil is inorganic and is heat stable. Conducive soil became suppressive to *P. capsici* after ignition at 500 C for 16 hr. It is not known if the suppression mechanism of ignited conducive soil is the same as that of natural suppressive soil.

Aqueous extract of suppressive soil was not inhibitory to germination of sporangia of *P. capsici* (Table 3). In another experiment, sporangia of *P. capsici* germinated 89% in the extract concentrated to one-fourth of its original volume by evaporation. These results suggest that the inhibitory factor is not water-soluble.

Amendment of soils with ethylenediaminedi-*o*-hydroxyphenylacetic acid or ethylenediaminetetraacetic acid at 1,000 μg/g did not increase sporangial germination in suppressive soil or decrease germination in conducive soil (Table 3). This result indicates that the suppression is not due to the presence or absence of certain chelatable ions. When sporangia of *P. capsici* were placed on a polycarbonate membrane (47-mm diameter, 0.2 μm, Nuclepore Co., Pleasanton, CA) on the soil, germination increased from 1% without membrane to 92% with membrane, which suggests that the inhibitory factor in the suppressive soil is not diffusible. The clay, silt, and sand fractions of the suppressive soil were inhibitory to *P. capsici* (Table 3). Results suggest that physical or chemical characteristics of the inorganic soil particles are responsible for the inhibition of sporangial germination of *P. capsici* in the South Hilo soil.

LITERATURE CITED

1. Aragaki, M. 1964. Relation of radiation and temperature to the sporulation of *Alternaria tomato* and other fungi. Phytopathology 54:565-569.
2. Aragaki, M., Mobley, R. D., and Hine, R. B. 1967. Sporangial germination of *Phytophthora* from papaya. Mycologia 59:93-102.
3. Hwang, S. C., and Ko, W. H. 1974. Germination of *Calonectria crotalariae* conidia and ascospores on soil. Mycologia 66:1053-1055.
4. Jackson, M. L. 1956. Soil Chemical Analysis—Advanced Course. Published by the author, Department of Soils, University of Wisconsin, Madison. 991 pp.
5. 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.
6. Katsura, K. 1971. *Phytophthora* Disease of Plants. Seibundo-Shinkosha, Tokyo. 128 pp.
7. Ko, W. H., Chang, H. S., and Su, H. J. 1978. Isolates of *Phytophthora cinnamomi* from Taiwan as evidence for an Asian origin of the species. Trans. Br. Mycol. Soc. 71:496-499.
8. Ko, W. H., Chase, L. L., and Kunimoto, R. K. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63:1206-1207.
9. 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.
10. Ko, W. H., and Hora, F. K. 1972. Identification of an Al ion as a soil fungitoxin. Soil Sci. 113:42-45.
11. Ko, W. H., and Hora, F. K. 1974. Factors affecting the activity of a volatile fungistatic substance in certain alkaline soils. Phytopathology 64:1042-1043.
12. Ko, W. H., and Lockwood, J. L. 1967. Soil fungistasis: Relation to fungal spore nutrition. Phytopathology 57:894-901.
13. Ko, W. H., and Nishijima, K. A. 1984. A Hawaiian soil suppressive to *Phytophthora capsici*. (Abstr.) Phytopathology 74:799.
14. Kobayashi, N., and Ko, W. H. 1985. Nature of suppression of *Rhizoctonia solani* in Hawaiian soils. Trans. Br. Mycol. Soc. 78:(In press).
15. Robinson, W. O. 1927. The determination of organic matter in soil by means of hydrogen peroxide. J. Agric. Res. 34:339-356.
16. U.S. Department of Agriculture. 1976. Soil Survey Investigations Report 29. Soil Conservation Service, USDA, Washington, DC.