

## Factors Affecting the Activity of a Volatile Fungistatic Substance in Certain Alkaline Soils

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### ABSTRACT

Hydrogen peroxide treatment of an alkaline soil decreased the organic matter content 90%, while more than 70% of the volatile fungistatic substance was retained. The volatile inhibitor existed in the fractions of sand, silt and clay, and did not completely disappear from soil after ignition at 400 C for 12 h. The amount of the volatile inhibitor released was directly correlated with the amount of water added. No volatile inhibitor was released from soil with the same moisture content as that under field conditions. Results suggest that the volatile inhibitor from certain alkaline soils is inorganic in nature, and that it is released under natural conditions only when the soil is wet, a situation which is not common in the regions where alkaline soils occur.

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Most fungal spores rest in soil without germination unless nutrients are provided (9). Such widespread soil fungistasis can be attributed to unavailability to spores of the exogenous and endogenous nutrients required for germination in soil, due to microbial competition (8). However, the fungitoxin in some acid soils (5) and the volatile inhibitor from certain alkaline soils (2, 7) may be

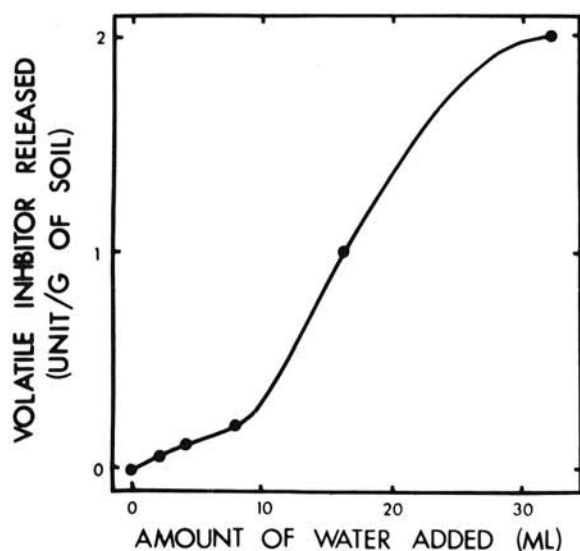


Fig. 1. Effect of soil moisture on release of the volatile inhibitor from Oahu soil. Various amounts of water were added to 50 g of natural soil which contained 8.3% moisture.

an additional factor contributing to suppression of spore germination in those soils. Although the fungitoxin was subsequently identified to be an Al ion (6), the volatile inhibitor still has not been identified. We reported herein some factors affecting the activity of the volatile inhibitor in certain alkaline soils.

**MATERIALS AND METHODS.**—The soil used was a dark magnesium clay (clay, pH 7.6) collected from the island of Oahu. In some experiments, Colorado swale soil (clay loam, pH 8.3) was also included for comparison. The moisture contents of Oahu and Colorado soils before use were 8.3% and 4.2%, respectively. The amount of organic matter in soil was decreased by H<sub>2</sub>O<sub>2</sub> treatment (10), and the organic matter content was determined by the Walkley-Black method (4).

The method of Jackson (3) was used for separating sand, silt, and clay from soil. One part of soil was mixed with ten parts of water (w/w) in a Waring Blender for 20 min. The soil suspension was passed through a 44- $\mu$ m sieve. 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 resulting sediment was used as the clay fraction.

The method for assaying the volatile inhibitor from soil was described in a previous paper (7) using *Penicillium frequentans* Westling as the test fungus. For quantitative determination of the volatile inhibitor in soil, spore germinations were tested on agar disks exposed to various amounts of soil, with moisture content adjusted to 70% waterholding capacity, in a petri dish (100 $\times$ 20 mm). One unit was defined as the amount of volatile inhibitor capable of reducing spore germination to 50% as determined from the dosage-response curve. Without the presence of the inhibitor, *P. frequentans* conidia germinated 90-100%.

**RESULTS AND DISCUSSION.**—The activity of the volatile inhibitor in eight samples of Oahu soil ranged from 0.1-1.0 units/g air-dried soil, while that in two samples of Colorado soil was 50 and 22 units/g soil. This was in accordance with a previous report (7) which showed that the volatile inhibitor released from Colorado soil was much stronger than that from Oahu soil.

When Oahu soil was treated with H<sub>2</sub>O<sub>2</sub>, the organic matter content decreased from 7.0-0.7%. However, the activity of the volatile inhibitor was decreased only from 0.7-0.5 units/g soil. The activity of the volatile inhibitor in the fractions of sand, silt, and clay was 0.2, 0.04, and 0.03 units/g, respectively. These results indicate that the volatile inhibitor may be an inorganic compound.

The activity of the volatile inhibitor decreased, but did not disappear from soils after their ignition at 400 C for 12 h, a procedure which has been used to thermally oxidize organic matter in soil (4). The activity decreased from 0.7 to 0.02 units/g of Oahu soil and 22 to 0.1 units/g of Colorado soil. The ignited Colorado soil still contained about the same amount of volatile inhibitor as one of the nontreated Oahu soil samples. This further indicates the inorganic nature of the volatile inhibitor.

To study the effect of soil moisture on release of the volatile inhibitor from soil, 0, 2, 4, 8, 16, and 32 ml of water was added to 50 g of Oahu soil. The amount of the

volatile inhibitor released was directly correlated with the amount of water added (Fig. 1). One hundred units of the volatile inhibitor were released from 50 g of soil when 32 ml of water was added. However, no volatile inhibitor was released from soil with the same moisture content as that under field conditions. When 100 g of Oahu soil in a beaker was kept moist in the greenhouse for 2 mo, the activity of the volatile inhibitor decreased from 0.6 units/g to a nondetectable level. Under the same conditions for 8 mo, the activity in Colorado soil also decreased from 22 to 0.3 units/g. Since alkaline soils are characteristic of arid and semi-arid regions (1), our results may explain why the volatile inhibitor can be easily detected in certain alkaline soils, but not in acid or neutral soils (2).

Our results suggest that the volatile inhibitor from certain alkaline soils is inorganic in nature and that it is released under natural conditions only when the soil is wet, a very infrequent situation in the regions where alkaline soils exist. This may account for the continuous existence of the volatile inhibitor in these soils.

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