MICROBIAL RESPONSES TO ORGANIC AMENDMENTS IN HOUSTON BLACK CLAY [1]

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INTRODUCTION

King (*5*)[3] and various colleagues in a series of papers reported the control of cotton root rot (*Phymatotrichum omnivorum* (Shear) Dug.) by the application of large amounts of stable manure to irrigated plots of alkaline soil in Arizona. Preliminary results, especially those obtained by the use of Cholodony slides (*6*), pointed to great increases in the microflora of the soil as accompanying, perhaps causing, the reduction in growth and activity of the root rot fungus. Since the same disease has produced losses in the cotton crop running into millions of dollars each year for over half a century in Texas (*3*), study of soil microbiological aspects of the problem under Texas conditions was necessary.

The soils infested with the root rot organism in Texas belong to the Houston series and related soil types. These soils constitute the black land prairie areas of Texas; they are alkaline in reaction, ranging from about pH 8 to 8.5 or even 8.7. They form a group of highly colloidal and close-textured calcareous types, with high moisture-holding capacity and with a tendency to become very hard and to crack in dry periods. Thus they present a pronounced contrast to the northern and eastern soils, which have formed the basis for most studies of the microbial floras of American soils.

Microbiological studies of Houston soils, exclusive of those dealing with the distribution of the *Phymatotrichum* fungus, have been limited. Werkenthin (*14*) isolated and identified some of the saprophytic molds of the Austin region; Morrow et al. (*8*) isolated and identified the mold flora of another area; Williams (*15*) noted some bacterial contents of virgin and cultivated soils; and Lewis (*7*) determined the distribution of green fluorescent bacteria in several Texas soils, including Houston clay. As a background for planning further experimentation with these difficult soils, a comparative study of microbial floras under environmental conditions presented by certain selected plots in cotton fields is reported here.

EXPERIMENTAL PROCEDURE

Field-soil samples were taken periodically from November 11, 1937, to December 15, 1938, from plots under different types of crop man-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 533.

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agement. Two of the three plots chosen for study were from an experiment designed for comparing the effect of turning under a second crop of sorghum with the effect of continuous cotton cultivation upon the incidence of root rot of cotton. These plots were located in Soil Fertility's [4] experiment No. 12 on Blank's plantation, near Lockhart, Tex. Sorghum residues, turned under on October 25, 1937, were estimated at about 3,000 pounds, dry weight, per acre. A third plot was chosen from another Soil Fertility experiment on the Voelker farm near Kimbro, Tex., on which cowpeas were grown in 1937 and the entire crop turned under on October 18, 1937. The area carrying cowpeas had not been selected for this experiment at the time the crop was turned, so the amount of material turned is not known. The growth was good. All three plots were cropped to cotton in 1938.

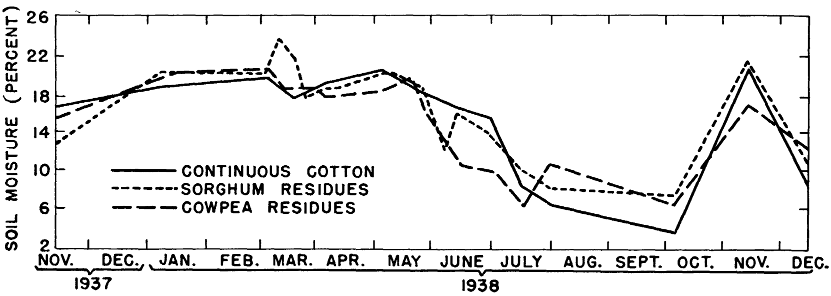

FIGURE 1.—Soil moisture, as determined by air-drying, for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

Soil samples were collected from approximately 4-inch and 12-inch levels; each soil sample was a thoroughly mixed composite of roughly equivalent soil portions taken from four separate locations. Soil and air temperatures were taken at the time of collection of field samples. Cultures were made from moist soils as soon after collection as practicable. The total number of micro-organisms was determined from Petri-dish cultures on sodium albuminate agar; the number of actinomycetes on glycerol nitrate-soil extract agar; and the number of filamentous fungi on acid-dextrose-peptone agar. Spores of aerobic bacilli were determined by plating out the appropriate dilutions on nutrient beef agar after pasteurization at 80° C. for 10 minutes. Quantitative estimates of blue-green fluorescent bacteria were made by the dilution-tube method, on an asparagine medium, and the most probable numbers were determined from the tables of Halvorson and Ziegler (*4*). Soil moisture (fig. 1) was determined by air-drying 100-gm. portions of moist soil; the observed loss in weight was expressed as percentage of moisture in moist soil.

RESULTS

Population changes for various microbial groups found in Houston black clay at different seasonal dates and under different conditions of soil management are presented graphically in figures 2 to 6 for the

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4-inch level of sampling. The results obtained from samples taken at the 12-inch level followed the same general course. The numbers of micro-organisms were conspicuously lower than those for the upper level. Although the figures were obtained and tabulated they are omitted here as not changing the significance of the studies made at the 4-inch level.

TOTAL PLATE COUNTS

The period of maximal activity for all plots, as indicated by the total counts (fig. 2), was found to begin in March and to end early in June. The greatest activity was shown in soil receiving organic residues. The highest total counts in the plots receiving sorghum residues were 428.4 million per gram of dry soil, and for plots receiving cowpeas, 430.7 million, whereas the largest population observed in the continuous-cotton soil was 179.4 million.

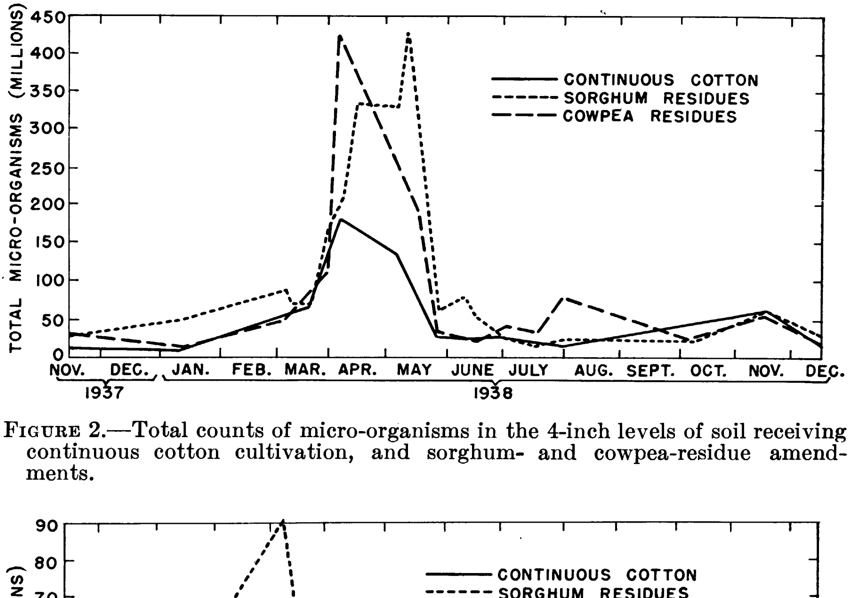

FIGURE 2.—Total counts of micro-organisms in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

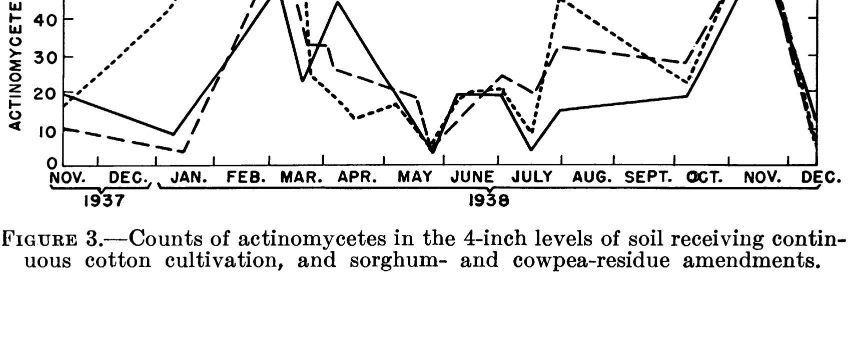

FIGURE 3.—Counts of actinomycetes in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

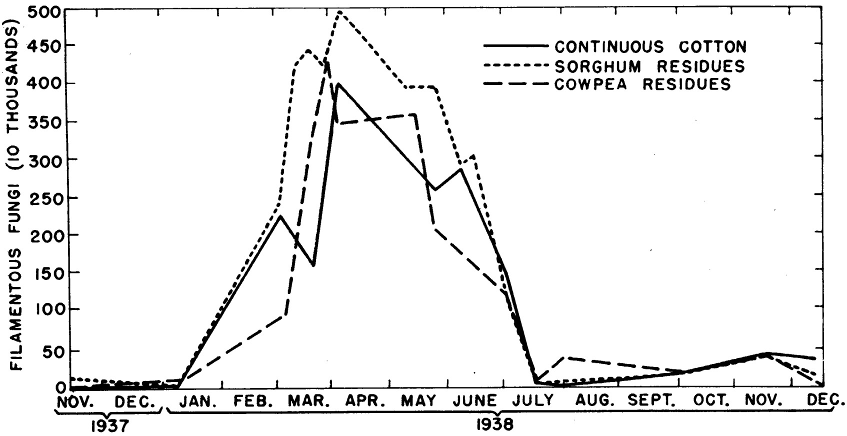

FIGURE 4.—Counts of filamentous fungi in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

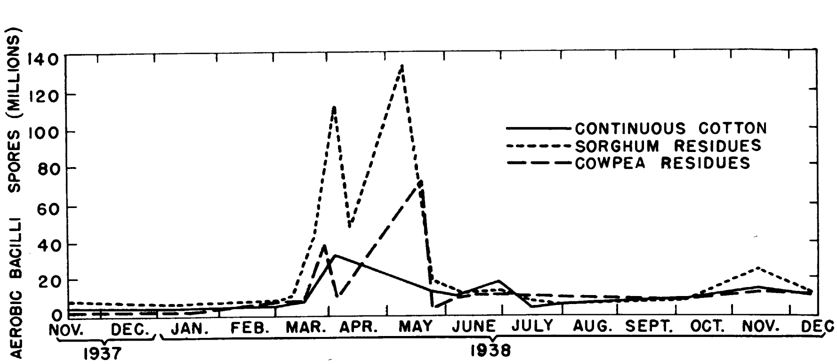

FIGURE 5.—Counts of aerobic bacillus spores in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

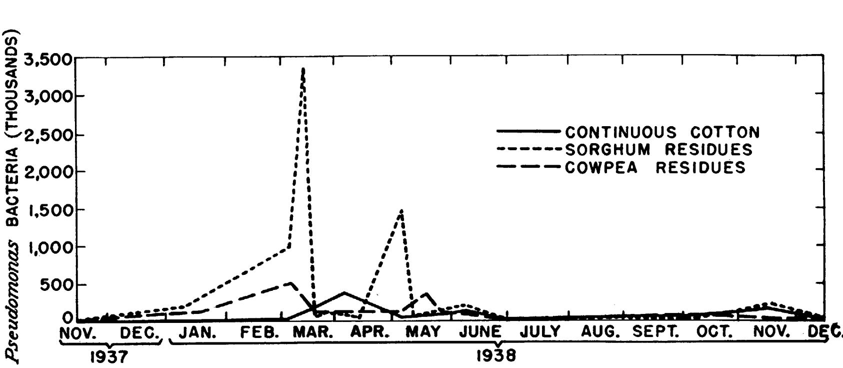

FIGURE 6.—Counts of *Pseudomonas* bacteria in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

The numbers of micro-organisms found in these samples of Houston black clay are greater than those generally reported in studies of soil. Waksman (*13*) has reported from 7 to 10 million total bacteria per gram for rich garden and cultivated orchard soils in New Jersey; Erdman's data (*2*) on Iowa Carrington loam reveal generally 7 to 15 millions per gram; and Vandecaveye (*12*) reports from 3 to 20 millions per gram in his studies on Palouse silt loams in Washington.

FUNGI

The fungi, like the bacteria, showed extensive increases during the spring months. This increase in fungus population became apparent earlier in the season and continued later into the summer than did that of other microbial populations (figs. 2 and 4). The number of fungi in the three soil treatments studied showed smaller differences according to type of soil management than did those of other groups of micro-organisms considered.

ACTINOMYCETES

Increased numbers of actinomycetes appeared during the late winter period in all plots (fig. 3). In early March, the colony count per gram of soil reached 47.5 million for the continuous-cotton soil, 89.5 million where sorghum residues were added, and 59.7 million where the cowpeas were plowed under. The fluctuations shown in colony count of actinomycetes have not been explained, but the totals involved are strikingly greater than those shown in previously reported studies (*2*, *13*).

In a subsequent experiment at the Greenville station, sorghum buried deeply in bundles over which cotton was planted was dug out in the last week of August 1939. As the area was broken open the odor of *Actinomyces* was detected strongly, and sorghum stalks only partly decomposed were powdery white with *Actinomyces* spores. In the buried bundles, the presence of open spaces or cracks was an obvious factor in the development of powdery spore masses. Temperatures in the soil, 10° to 20° higher than those found in northern and eastern soils, may be an important factor in the totals found.

SPORES OF BACILLI

Changes in the number of spores of aerobic bacilli (fig. 5), under different seasonal conditions and under different types of soil management, parallel roughly the changes observed for total microbial populations. Maxima were obtained during the spring period; thus the effect of organic residues was apparent. The maxima for unamended soil and for treatments with sorghum and cowpea residues were 31.4 millions, 132.4 millions, and 72.5 millions, respectively.

The blue-green fluorescent bacteria (fig. 6) showed response to added organic residues during the latter part of the winter. Maxima observed for the sorghum- and the cowpea-residue amended soil were 3,400 thousands and 500 thousands, respectively, and for the unamended soil, 370 thousands. The first two values were observed in early March, and the last in early April. Like total bacteria and sporulating bacteria, *Pseudomonas* types showed depressed numbers during the summer and early fall months.

DISCUSSION AND INTERPRETATION OF RESULTS

Study of figure 7, showing the general temperature relations through 1 calendar year, shows that soil temperatures at the 4-inch level fell below 60° F. about December 1, 1937, and again reached 60° early in February, and 70° by March 1, 1938. At no time were freezing temperatures observed. Nevertheless, the cooler temperature shown would readily account for the fact that the total number of micro-organisms was static (fig. 2) from the latter part of November to the

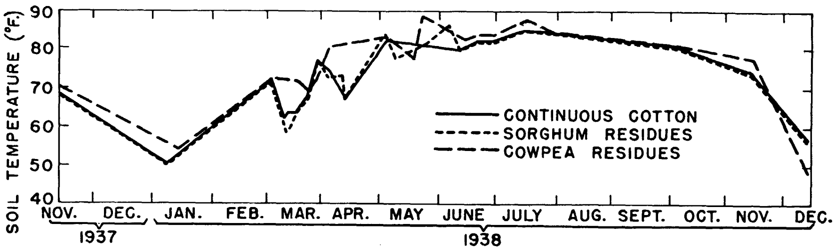

FIGURE 7.—Soil-temperature readings for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

early part of March. Very low moisture content in the latter part of July to October clearly correlates with low bacterial activity during that period. Such observations are in entire harmony with the observations of many workers (*1, 2, 4, 9, 13*).

Comparison of figures 1, 2, and 7 shows a rather sharp fall of temperatures beginning about November 1, 1937, accompanied by small changes in bacterial population, as indicated by the colony count in Petri-dish cultures. Although there was a differential rise in number of actinomycetes during the winter months (fig. 3), the total flora as shown by colony count (fig. 2) changed little before the temperature rose to 70° F. again early in March. The abrupt maxima reached in April and May can therefore be interpreted as the resumption of bacterial decomposition in the organic matter plowed under late in October. This activity would appear to have been arrested by the falling temperatures. The peak number for the 4 spring months, reaching more than 400 million micro-organisms, represents the normal rise where such amounts of plant materials are plowed under (compare Smith and Humfeld, *10, 11*). It is noteworthy that in the plot receiving the residues from the previous cotton crop alone, the rise was simultaneous, even though the highest figure was somewhat under half (179 millions) of that for the green crops plowed under.

Transferred to Texas conditions, King's hypothesis that the introduction of masses of organic matter would produce great microbial activity is fully confirmed. The numbers reported here from colony counts in Petri-dish cultures are strikingly higher even than those of Smith and Humfeld (*10, 11*) from their composite samples.

SUMMARY

Curves for temperature, moisture, and microbial numbers were established for selected plots of Houston soil as a background for

studies in root rot control. As an environment for microbial activity, the plots of Houston soil studied furnish the following striking features:

(1) In a period between early December and February the temperatures remained below those required for active microbial multiplication, yet without freezing.

(2) Temperatures ranged above 70° F. from March to November, and from 80° to 90° from June to October.

(3) Bacteria and actinomycetes are much more active and abundant than has been reported for northern soils. Maxima for total colony counts in soils receiving organic amendments reach 200 to 400 millions; actinomycetes at times reach 50 and 90 millions to the gram. Such responses to added organic nutrients present a challenge to the worker to search for organic media and agronomic practices capable of yielding a controlled microflora.

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