AN INVESTIGATION OF POPULATIONS OF AEROBIC SPOREFORMING BACTERIA IN THE SOIL

by

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INTRODUCTION

The ecology of the microflora of soils is far less clear cut than that of populations of some other environments. It appears that microbial populations are dependent upon environmental conditions, available energy materials, and possible competition between different types of organisms. An organism must possess unusual characteristics in order to survive in such a complex environment. The sporeforming Bacillus has the unique characteristic of being able to survive in adverse environmental conditions, and has the ability to resist inhibitory substances. Also, the genus Bacillus is considered zymogenous in nature since members of this group become very active when organic nutrients are added.

These sporeforming bacteria of the soil do not decrease in numbers and spores cannot live forever. Their occurrence in soil cannot be due to accidental contamination or their numbers would not be so constant (Conn, 1916).

Since most investigations have been concerned with populations under specific conditions, present experiments were aimed at determining the population variance over a period of time and under different conditions of moisture, temperature, and available nutrients.

REVIEW OF LITERATURE

Alexander (1961) stated that five to twenty per cent of the organisms of the A horizon are strains of Bacillus. Colonies that have developed on normal plate counts will not only indicate viable counts, but will

also detect a colony that has developed from a spore. In areas not recently amended with organic matter, Bacillus is probably found in the spore state, persisting in the dormant condition for many years. However, when specific nutrient conditions are provided they become active, and as a result of the rare population burst the soil becomes inhabited for many years by the dormant endospores.

Most prominent among the sporeforming bacteria in the soil are the strongly proteolytic species B. cereus, B. mycoides, B. megaterium, and one or two others (Conn, 1948). According to Conn (1916), the numbers of sporeforming bacteria in soil are relatively constant and are about the same in all soils studied. The total number of B. mycoides, B. cereus, and B. megaterium, as determined by means of gelatin plates, proved to be between 400,000 and 1,500,000 per gram in the soils studied, usually about five to ten per cent of all colonies developing.

Conn's data (1916) suggested that these bacteria occur in normal soil as spores rather than in a vegetative state. His procedure to determine this was as follows: when soil infusions were heated, before plating, at a temperature (75-85 C) high enough to kill the vegetative forms of bacteria, nearly as many colonies of these sporeforming bacteria developed as when it was plated unheated. In about one third of the cases, their numbers were actually slightly higher on the plates made after heating. Also, when fresh manure was added to a pot of soil, no increase or decrease in the total number of sporeformers was detected.

According to Conn (1916), it stands to reason that these bacteria so universally present in the soil must grow and multiply under some

natural conditions. The results noted throw considerable doubt on the assumption that these organisms are important ammonifiers in the soil. It is known that they ordinarily thrive in the presence of organic matter but not in the case of manure added to the soil. It is plain that ammonification can take place without them.

Several workers have noted conditions in which Bacillus strains have been associated with plant roots or in the rhizosphere. Clark (1940) showed that sporeforming rods, or species of Bacillus, are depressed in the rhizosphere. He demonstrated that the relative frequency of occurrence of certain Bacillus species is different on root surfaces than in the soil. It was shown that Bacillus numbers are appreciably depressed on root surfaces, perhaps to one tenth their numbers in soil. A selective encouragement of a few was also noted. The few encouraged are B. polymyxa, B. brevis, and B. circulans. They were encountered in greater relative abundance within the genus Bacillus.

Lochhead (1940) investigated the relative incidence of bacterial types occurring in the rhizosphere of different plants and in control soils. The study indicated that the qualitative nature of the soil microflora is markedly influenced by the growing plant. In the rhizosphere, Gram-negative rods are proportionately increased while Gram-positive rods, coccoid rods, and sporeforming types are relatively less abundant.

Lochhead et al. (1940) further showed that sporeforming types were relatively less numerous in the rhizosphere than in soil more distant from the plant. He found that a selective action was characteristic of the rhizosphere of all plants studied, namely clover, oats, flax,

corn, and tobacco.

Later work by Clark and Smith (1949) showed that certain species of Bacillus constituted a relatively larger fraction of the spore-forming population present on wheat roots than of that present in the soil. It was demonstrated that numerous species of Bacillus grow and multiply upon the roots of plants grown in quartz sand given only mineral nutrients. Under such conditions, aerobic sporeformers are present in much higher numbers in the rhizosphere than they are in the sand apart from the roots. Their numbers are found to increase during a considerable portion of the period of plant growth.

Isolation and identification of dominant colony types revealed that sporeforming flora which developed on plant roots was dissimilar from that commonly encountered in field soil. There was a greater percentage of B. brevis and B. circulans in the rhizosphere than in soil apart from plant roots. However, organisms such as B. cereus, B. megaterium, and B. subtilis were quite commonly isolated from the surface of plant roots (Clark and Smith, 1949).

Clark and Smith (1949) also noted that in laboratory soil receiving peptone but not added sugar, B. sphaericus and related species became dominant. This confirms the hypothesis that for the most part they are inert, and only for short intervals of time do they find conditions for their development. However, the association of B. brevis and B. circulans groups with plant roots, and the observation of significant differences in the number of spores associated with the roots of different plants, suggest that there are certain conditions in soil, of fairly long duration, to which certain species of Bacillus respond

in much the same manner as do non-sporing bacteria.

Steinberg (1951) reported the occurrence of large populations of B. cereus in the rhizosphere of frenched tobacco. However, he did not demonstrate a causal relationship between the bacteria and the disease.

Antagonistic effects by other soil microflora may also influence the population of certain Bacillus species. Conn and Bright (1919) showed an antagonistic effect between B. cereus and Pseudomonas fluorescens. They inoculated sterilized manured soil with a mixture of B. cereus and Ps. fluorescens cells and made subsequent quantitative analysis for a period of fifteen days to determine the relative rate of multiplication. They failed to obtain colonies of B. cereus while Ps. fluorescens multiplied enormously. Later work by Lewis (1929) showed that Ps. fluorescens produces toxins in sterilized manured soils. The toxins produced were extracted by alcohol and were shown to inhibit the growth of B. cereus and B. mycoides on nutrient agar.

Mitchell and Alexander (1963) have demonstrated the lysis of Fusarium oxysporium by B. cereus. They noted that living and dead Fusarium mycelium as well as cell-wall preparations were digested by this bacterium. Their evidence suggests that lytic microorganisms can destroy fungal mycelium in sterile soil.

Egeberg et al. (1964) demonstrated strong antagonists to Coccidioides immitis. B. subtilis was found to be one of the antagonists isolated from the soil. They concluded that C. immitis, a natural inhabitant of the soil, lives in a state of balance with many other organisms, and that the weather affects the population

of the organisms, among them C. immitis and its antagonists.

Antibiotic producing organisms will show some form of antagonism against Bacillus species. Siminoff and Gottlieb (1951) demonstrated that some form of antagonism developed between Streptomyces griseus and B. subtilis to the detriment of the bacterium. However, it was noted that this antagonism could not be the result of the secretion of streptomycin since it is inactivated in the soil by a highly irreversible absorption. Furthermore, a similar effect was demonstrated by a non-streptomycin-producing mutant. By contrast, an investigation by Gottlieb and Siminoff (1952) showed that chloromycetin, synthesized by Streptomyces venezuelae, was unable to antagonize the growth of B. subtilis in unamended soil.

Bacterial numbers have been shown by Stevenson (1962) to increase during the decomposition of plant residues. Bacterial numbers reached their peak during early stages of decomposition. In view of the tremendous increase in numbers during the first week, it was of interest to determine what groups of organisms made up the "zymogenic" population. Nutritional grouping of the bacterial isolates of the soil with added flax residues indicate a definite increase in the numbers or organisms which have relatively simple nutritional requirements such as basal salts or amino acids. These would fit into the classification of Lochhead and Chase (1943) in which organisms isolated from the soil with simpler requirements consisted, to a larger extent, of sporeforming rods and Gram-negative nonsporing rods.

Other publications have related Bacillus to soil aggregation. Martin (1945) described an aerobic bacillus, apparently belonging to

the B. subtilis-mesentericus group, that brought about marked aggregation of the silt and clay particles of the soil. A hemicellulose-like polysaccharide synthesized by the organisms was found to be primarily responsible for the marked aggregating effect. It was found that the active aggregating material was attacked, to a limited extent, by fungi but was readily destroyed by certain bacteria and actinomycetes. It, therefore, belongs to the class of microbial aggregating agents which only temporarily contribute to increased soil aggregation.

Finally, Mahmoud (1957) studied germination, growth, and sporulation rate of B. subtilis and B. cereus spores in sterile soil with different moisture content. He found that the extent of growth and sporulation of B. subtilis and B. cereus in flooded soil is less than in soil with twelve per cent moisture. The phenomenon was attributed to the dilution of nutrients in flooded soil or to reduced aeration, or both acting together. The results showed that the population stabilized and remained stable throughout the experimental period.

The literature, in general, indicates that the aerobic sporeforming populations in the soil can be affected by the conditions in the rhizosphere, the competitive and antagonistic environments, and the decomposition of plant residues. Any combination of these complex factors would either stimulate a higher population or would have a detrimental effect against the organisms. With these different conditions in mind, it becomes of interest to introduce different environmental conditions such as moisture levels, temperature, and available nutrients to determine their effect on population variations.

MATERIALS AND METHODS

An assortment of four soils of differing characteristics were chosen for use in these experiments. All samples were taken from the upper two inches of soil.

The soils selected were:

Soil A Geary Silt Loam, field soil, intensively cultivated.

Soil B Geary Silt Loam, field soil, cultivated.

Soil C Alluvial Deposit, field soil, cultivated.

Soil D Sarpy Fine Sandy Loam, relatively low organic matter.

Table 1. Soil analysis.

Soil	% of Organic Matter	pH	Water Holding Capacity % Moisture
A	1.9	5.3	65
B	1.9	5.2	70
C	2.6	6.3	75
D	0.3	6.6	37

The moisture holding capacity of the soil was determined by first oven drying the soil. To 40 grams of dry soil, 10 ml of water were added which resulted in 50 grams of moist soil or soil with 25% moisture on a dry weight basis. This known quantity of moist soil was placed in a funnel into which had been fitted a cone of moist filter paper. One hundred ml of water were then filtered through the soil and collected in a graduated cylinder. The amount of water retained plus the known

amount of water in the moist soil represented the moisture holding capacity.

Total counts were carried out by standard dilution plate count methods. Eleven gram samples of soils were used. Incubation was at 30 C. for twenty-four hours. For spore enumeration, a dilution of the original soil suspension was pasteurized for fifteen minutes at 80 C. before plating. After pasteurization, standard dilution plate count methods were employed to determine the number of spores present. The plating medium used was nutrient agar (Difco) and plate counts were made in triplicate. The log of the number of bacterial cells per gram of soil was plotted against time in days to obtain a curve showing the population variance under different soil conditions.

Moisture content of the soils was measured by comparing the weights of ten gram samples before and after drying at 125 C for 24 hours. The following formula was used for calculations:

% moisture = grams lost during drying/weight of dry soil.

Soil samples were collected for a period of ten months to observe population variables in actual field conditions. Total counts, spore counts, and moisture determinations were made immediately after collection.

Soils for air drying were collected and placed in a 600 ml beaker. The soils were stored at room temperature, in a partially covered beaker and stirred at intervals to allow uniform evaporation. Total counts, spore counts, and moisture determinations were made throughout the experimental period.

Soils for refrigeration were collected, placed in covered 600 ml beakers and stored at 7 C. Total counts and spore counts were made as for other samples.

Peptone (Difco) was added to soil C in order to stimulate the normal population. The soil was collected and sieved through a ten mesh screen before making total counts, spore counts, and moisture determinations. Five milliliters of 10% peptone solution and 0.5 gm. peptone were added drop by drop, with constant stirring, to each one hundred grams of soil. After five days at room temperature, the usual counts were made. The sample was then divided in to three parts to provide:

1. A sample to be dried.
2. A sample to be kept moist in a plastic bag.
3. A one hundred gram sample to be dried prior to addition of nutrients.

Total counts, spore counts, and moisture determinations were made throughout the experimental period. Due to the absence of evaporation in a plastic bag, moisture determinations were not made on sample 2. Five milliliters of a 10% dextrose solution were added to Sample 3 twenty days after the initial addition of peptone.

Soil C was also used for pure culture inoculation. B. megaterium was isolated from the soil and identified according to Bergey's Manual of Determinative Bacteriology, 7th Edition (Breed et al., 1957). The organism for inoculation was grown in quantity on nutrient agar in large bottles. At the end of forty-eight hours, the cells were suspended in distilled water and washed three times. After washing, they were placed

in a 500 ml volumetric flask and diluted to volume. The total number of bacteria in the flask was estimated by taking optical density readings of the cell suspension at a wave length of 550 mu using a Bausch and Lomb Spectronic 20 spectrophotometer and comparing these readings to a calibration curve previously prepared. The amount needed for inoculation was calculated from the normal total counts of the soil and the number of cells in suspension. The total number of milliliters of cell suspension needed was poured into tubes and concentrated by centrifugation. The concentrated cell suspension was then added to the soil, drop by drop, with constant stirring. The organism was added to the soil in quantities of ten times the population and two times the population. After inoculation, the following determinations were made:

1. Total population and spore population at decreasing moisture levels.
2. Total population and spore population at sixty per cent of the moisture holding capacity.

Sixty per cent moisture holding capacity was maintained by adding water as needed to restore samples to their original weight. Samples were weighed at 48 hour intervals. The same procedure of inoculation was followed with B. cereus.

B. cereus and B. megaterium were also used for filter paper inoculation. This experiment was performed to compare the populations out of their natural environment. Each was inoculated separately on a square of Whatman No. 1 filter paper 15 mm on a side. The number of organisms added was determined by standard dilution plate count methods.

A 0.1 milliliter solution was placed on each filter paper. The total population and spore population was followed by placing one filter paper in a 100 ml dilution bottle and subsequently determining the number by standard plate count methods.

A population survey of sporeformers was set up on soils collected between 1959 and 1960. These soils were collected from different parts of the United States on pipeline surveys by Dr. J. O. Harris, Kansas State University, Manhattan, Kansas, and stored at room temperature in steel barrels. All samples were collected from the upper layer of the soil. The date, type of soil, and location are shown in Table 2.

Table 2. Identification of soils collected on pipeline surveys.

Date Collected	Soil Number	Type of Soil	Area of Collection
6-23-60	9-61	Clay loam	Southwest Wyoming
2- 9-60	1-11	Rocky loam	South Central Texas
4- 4-60	4-71	Sandy loam	Western Texas
5-17-60	7-31	Clay	Central California
11-16-59	M-31	Clay loam	Gulf coast of Texas
9-18-59	K-91	Clay	Central Mississippi
9-17-59	K-46	Clay	Western Arkansas
9-16-59	K-21	Sandy clay	Central Arkansas

A similar survey of sporeformers was set up on two different soils, clay loam and sandy loam, collected in 1926. These soils were collected in Russia by Dr. P. L. Gainey, Kansas State University, Manhattan, Kansas, and stored in a small rubber-stoppered bottle. Eleven grams of each soil were suspended in a 99 ml water blank. After suspension, total populations and spore populations were then determined.

RESULTS

Population Studies in Field Conditions

The results obtained in this study were used as a basis of comparison for later experiments in controlled conditions. The total population and spore population of soils B, C, and D failed to show marked variation over the period of time studied (Plates I, II, III). However, soil A (Plate IV) did not show the same stability as soils B, C, and D.

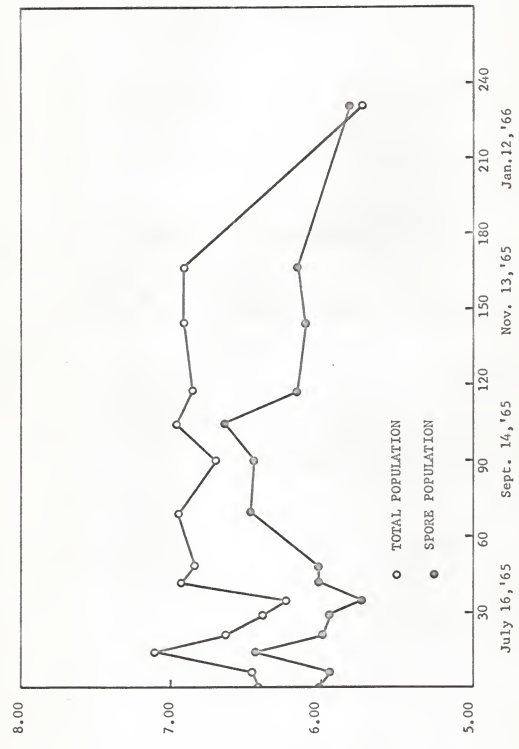
Population Studies at Decreasing Moisture Levels

Plates V, VI, VII, and VIII represent total and spore populations at decreasing moisture levels in the four soils previously described. The moisture content, at the time of the initial collection, was relatively high. The second sampling, five days later, showed an approximate forty per cent drop in moisture except for soil D which showed an eighty-eight per cent drop in moisture. The population survey at this point showed a decrease in the number of spores. The total population showed an increase in soils B, C, and D, and a decrease in soil A. After fifteen days, both the total and spore populations stabilized and remained

EXPLANATION OF PLATE I

Total populations and spore populations
of soil B during repeated collections.

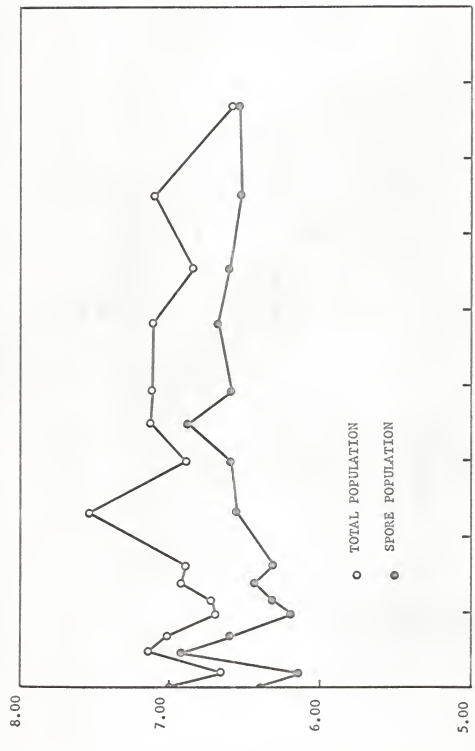
PLATE I


LOG NUMBER PER GRAM OF SOIL
8.00
7.00
6.00
5.00
TOTAL POPULATION
SPORE POPULATION
30
60
90
120
150
180
210
240
July 16,'65
Sept. 14,'65
Nov. 13,'65
Jan.12,'66
TIME (days)

EXPLANATION OF PLATE II

Total populations and spore populations of soil C during repeated collections.

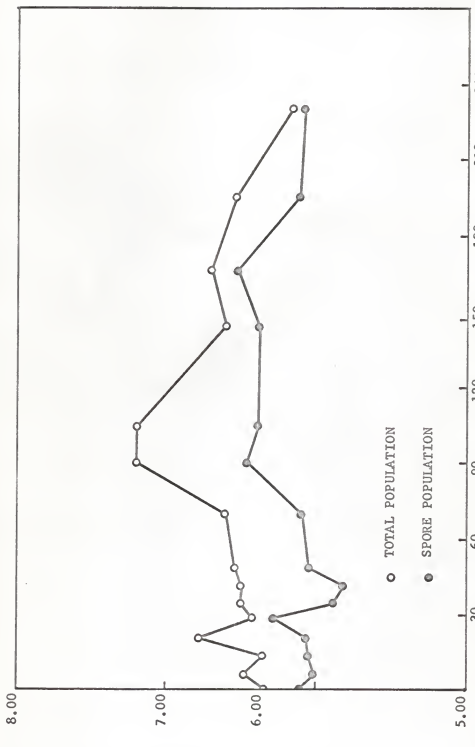
PLATE II


LOG NUMBER PER GRAM OF SOIL
8.00
7.00
6.00
5.00
30
60
90
120
150
180
210
240
TOTAL POPULATION
SPORE POPULATION
Jul16 16,'65
Sept. 14,'65
Nov. 13,'65
Jan. 12, '66
TIME (days)

EXPLANATION OF PLATE III

Total populations and spore populations of soil D during repeated collections.

PLATE III


LOG NUMBER PER GRAM OF SOIL
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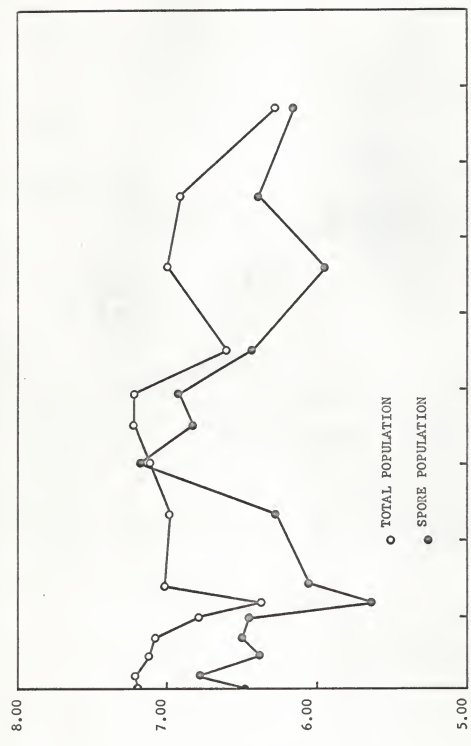
TOTAL POPULATION
SPORE POPULATION
30
60
90
120
150
180
210
240
July 16,'65
Sept. 14,'65
Nov. 13,'65
Jan. 12, '66
TIME (days)

EXPLANATION OF PLATE IV

Total populations and spore populations of soil A during repeated collections.

PLATE IV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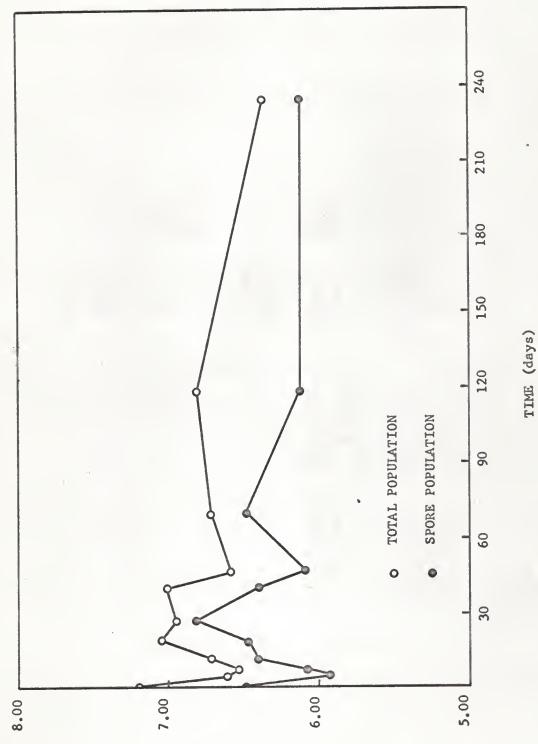
6.00
5.00
TOTAL POPULATION
SPORE POPULATION
30
60
90
120
150
180
210
240
July 16, '65
Sept. 14, '65
Nov. 13, '65
Jan. 12, '66
TIME (days)

EXPLANATION OF PLATE V

Total populations and spore populations of soil A at decreasing moisture levels.

PLATE V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LOG NUMBER PER GRAM OF SOIL
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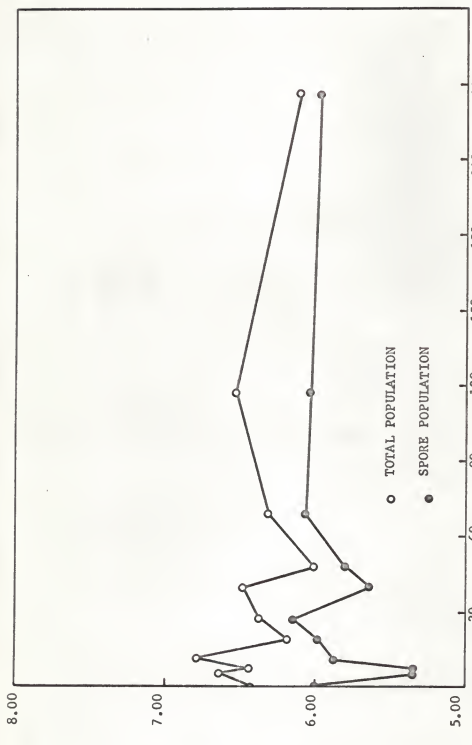
210
240
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE VI

Total populations and spore populations
of soil B at decreasing moisture levels.

PLATE VI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LOG NUMBER PER GRAM OF SOIL
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90
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210
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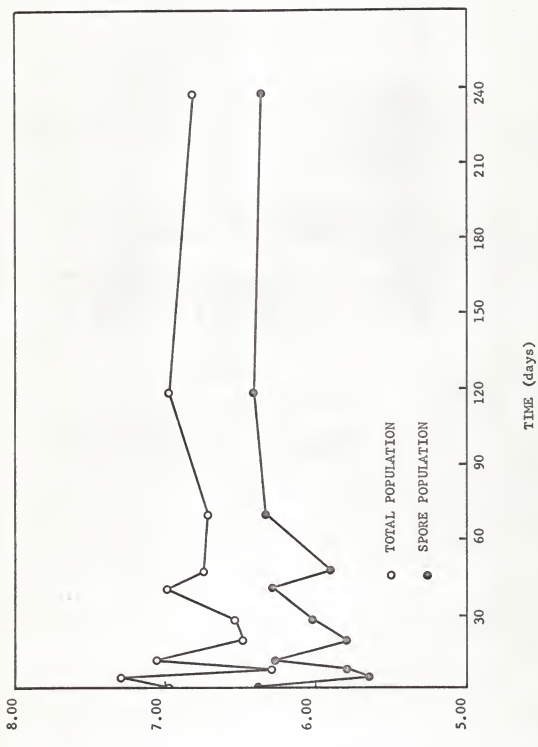
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE VII

Total populations and spore populations of soil C at decreasing moisture levels.

PLATE VII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120
150
180
210
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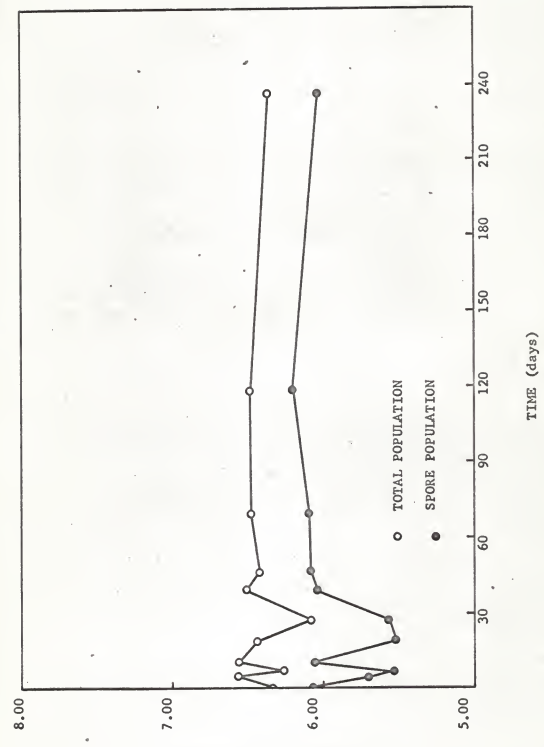
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE VIII

Total populations and spore populations
of soil D at decreasing moisture levels.

PLATE VIII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210
240
TIME (days)
TOTAL POPULATION
SPORE POPULATION

stable throughout the experimental period. The final moisture content varied from 0% in soil D to 3.20% in soil B.

Population Studies of Soil at 7° C

The populations of soils A, B, C, and D, at 7° C are represented by Plates IX, X, XI, and XII. Samplings, at five and seven days after the initial collection, showed a decrease in the spore population in the four soils studied. The total population in soils A, B, and D showed a proportional decrease in number as in the spore population. During the same period, the total population remained stable in soil C. The total population and spore population stabilized after fifteen days. Soil A and soil B showed the greatest stability throughout the experimental period.

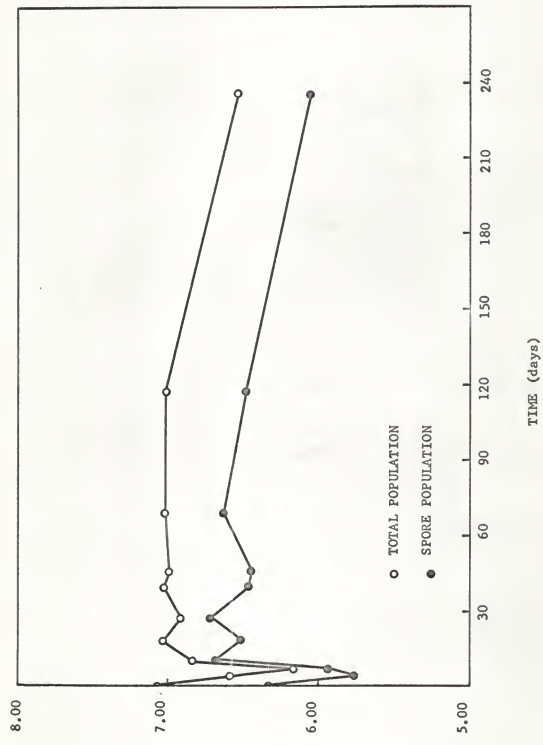
Population Studies in the Presence of Organic Additives

Soil C was chosen for the addition of peptone. The experiment was set up to stimulate a higher population and to observe any population variables after the zymogenous burst. Plate XIII represents the soil in a drying condition. A portion of the soil was placed in a plastic bag to retain the initial moisture level (Plate XIV). Both conditions showed parallel population trends. During the test period, neither the total population nor spore population fell below the initial normal population. Dextrose was added to a separate sample, twenty days after the peptone addition, to stimulate an additional population burst. However, as shown in Plate XV, the population increase was small. The next sampling showed a decline in numbers which then stabilized at that point

EXPLANATION OF PLATE IX

Total populations and spore populations of Soil A at 7°C.

PLATE IX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LOG NUMBER PER GRAM OF SOIL
30
60
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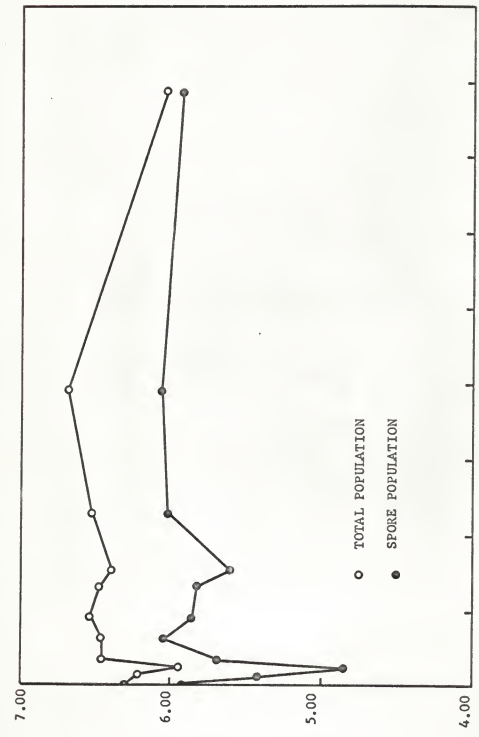
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE X

Total populations and spore populations of soil B at 7°C.

PLATE X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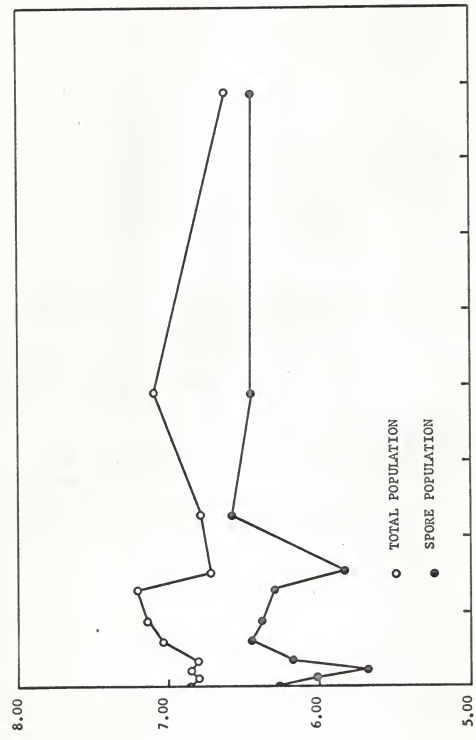
4.00
LOG NUMBER PER GRAM OF SOIL
30
60
90
120
150
180
210
240
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XI

Total populations and spore populations of soil C at 7°C.

PLATE XI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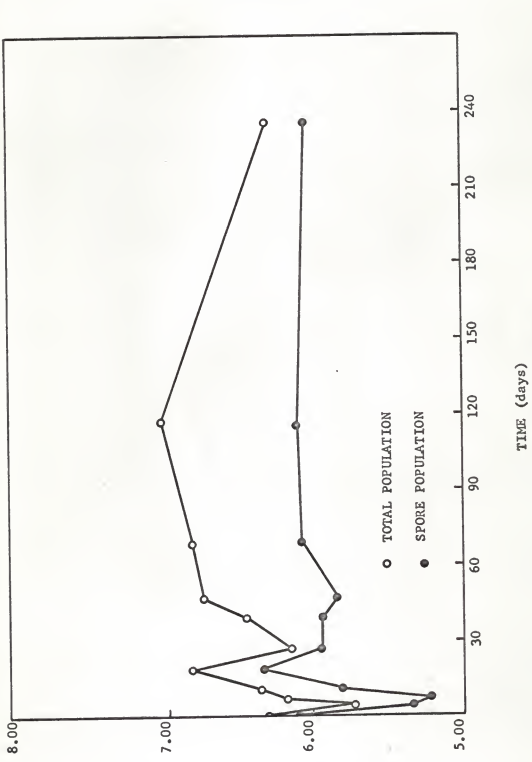
LOG NUMBER PER GRAM OF SOIL
30
60
90
120
150
180
210
240
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XII

Total populations and spore populations of soil D at 7°C.

PLATE XII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6.00
5.00
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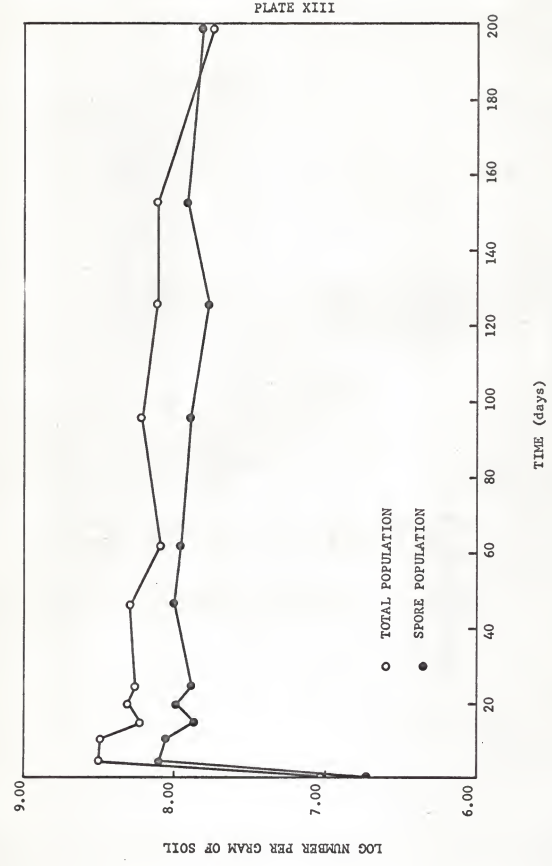
60
90
120
150
180
210
240
TOTAL POPULATION
SPORE POPULATION
TIME (days)
LOG NUMBER PER GRAM OF SOIL

EXPLANATION OF PLATE XIII

Total populations and spore populations of soil C, with the addition of peptone, at decreasing moisture levels.

PLATE XIII


LOG NUMBER PER GRAM OF SOIL
9.00
8.00
7.00
6.00
20
40
60
80
100
120
140
160
180
200
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XIV

Total populations and spore populations of soil C, with the addition of peptone, in a plastic bag to retain the initial moisture level.

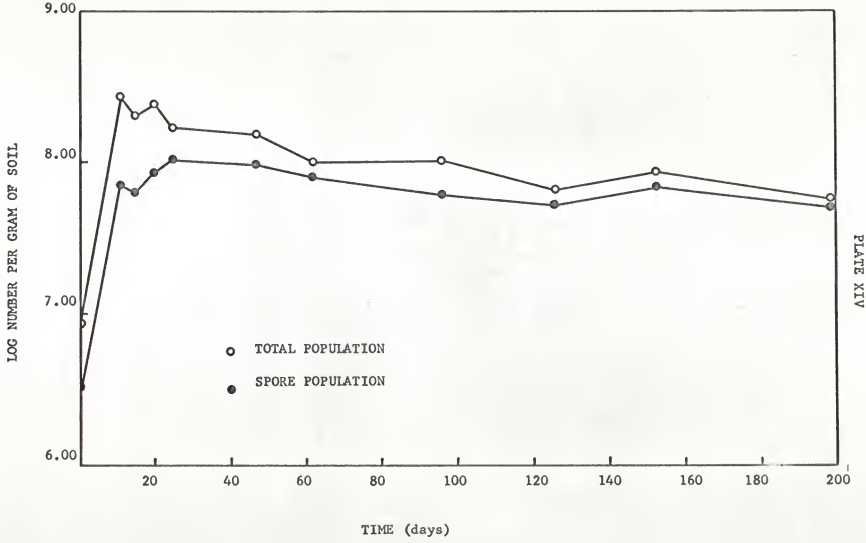
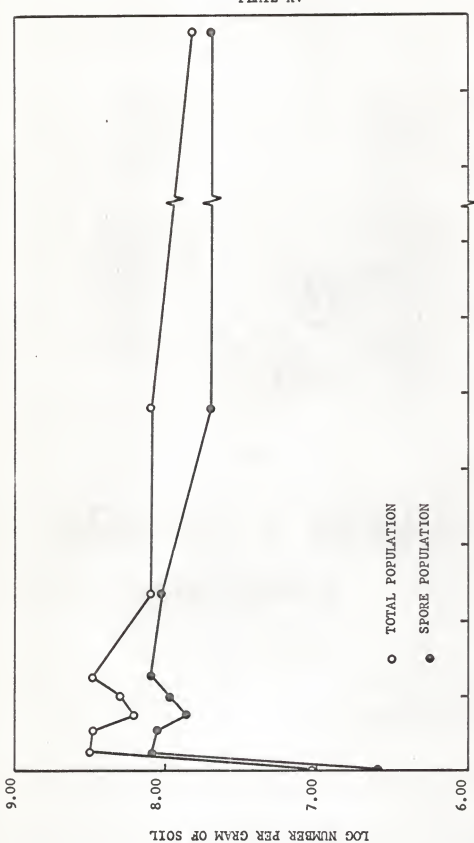

9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
TOTAL POPULATION
SPORE POPULATION
20
40
60
80
100
120
140
160
180
200
TIME (days)

PLATE XIV

EXPLANATION OF PLATE XV

Total populations and spore populations of soil C, with peptone and dextrose additions, at decreasing moisture levels.

PLATE XV


LOG NUMBER PER GRAM OF SOIL
9.00
8.00
7.00
6.00
20
40
60
80
100
120
140
260
280
300
TIME (days)
TOTAL POPULATION
SPORE POPULATION

and remained stable throughout the experimental period.

Population Studies of Inoculated Soil

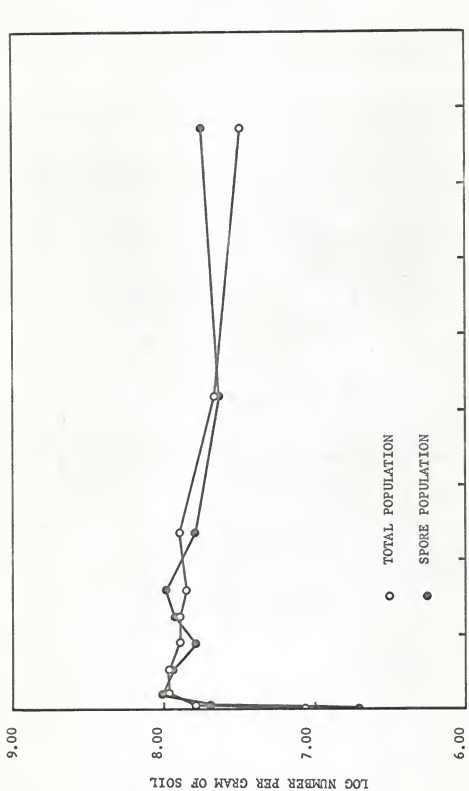
The pure culture inoculation experiment was set up to observe population trends of B. cereus and B. megaterium in their natural environment. Since the inoculated populations were ten times and two times the normal population, countable plates would only represent the inoculated population and not the normal population due to the high dilution factor. The natural condition of the soil would rule out any toxic effects which may arise from sterilized soil. Plates XVI and XVII represent B. cereus at two and ten times the population respectively. These plates represent the soil at decreasing moisture levels. At two times the normal population, spore counts were actually higher in several places than total counts. By contrast, the total count was always well above the spore count at ten times the normal population. Both demonstrated population stability during the experimental period. B. cereus showed a population decline in the same soil at sixty per cent moisture holding capacity (Plates XVIII and XIX). The phenomenon of higher spore population than total population occurred in both the two times the normal population sample and ten times the normal population sample. A similar observation was made by Mahmoud (1957) and Conn (1916).

Plates XX and XXI represent B. megaterium in drying soils at two and ten times the population respectively. B. megaterium, at two times the normal population, demonstrated a more stable population than at ten times the normal population. The organism showed a decrease in total population and spore population up to thirty days after inoculation

EXPLANATION OF PLATE XVI

Bacillus cereus, at two times the normal population, in soil C at decreasing moisture levels.

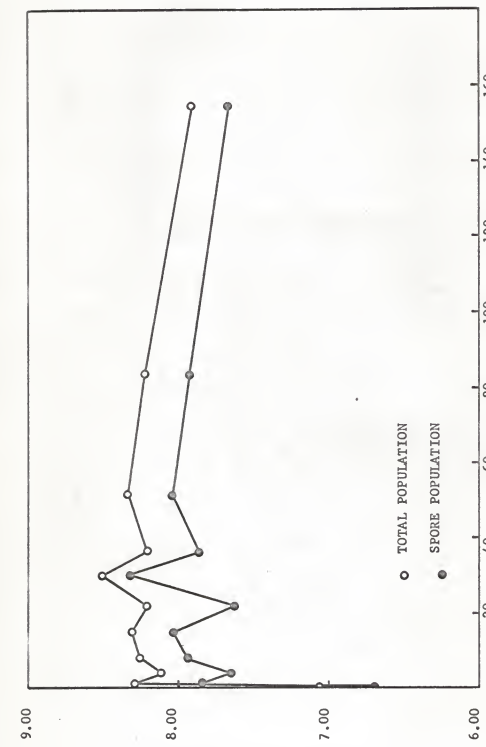
PLATE XVI


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XVII

Bacillus cereus, at ten times the normal population, in soil C at decreasing moisture levels.

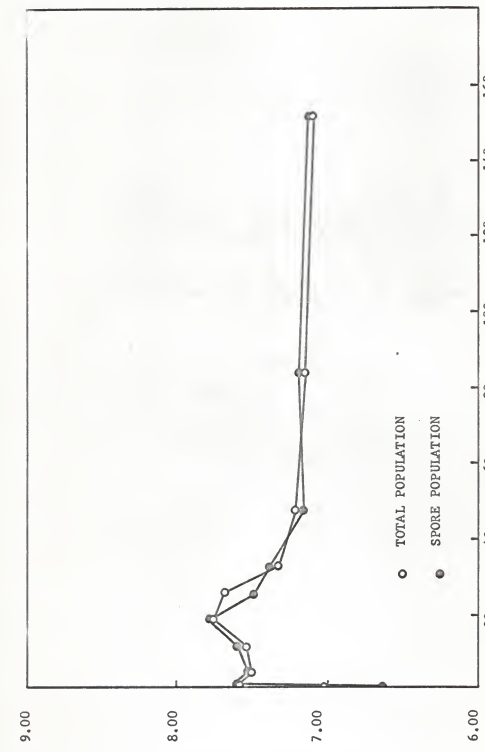
PLATE XVII


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XVIII

Bacillus cereus, at two times the normal population, in soil C at 60% of moisture holding capacity.

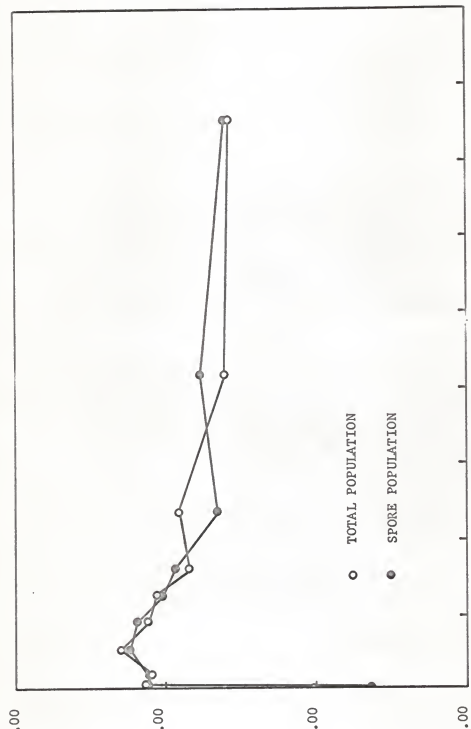
PLATE XVIII


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XIX

Bacillus cereus, at ten times the normal populations, in soil C at 60% of moisture holding capacity.

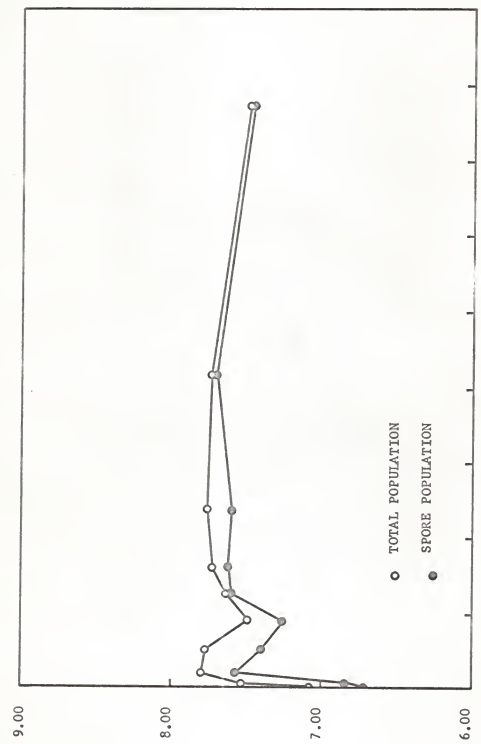
PLATE XIX


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XX

Bacillus megaterium, at two times the normal population, in soil C at decreasing moisture levels.

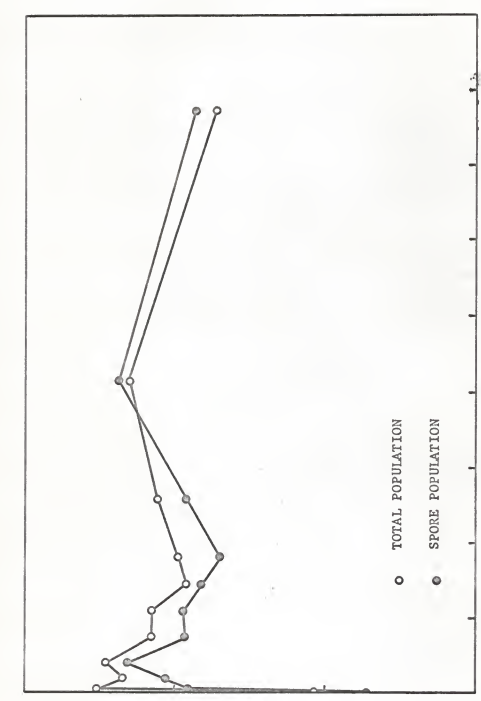
PLATE XX


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XXI

Bacillus megaterium, at ten times the normal population, in soil C at decreasing moisture levels.

PLATE XXI


9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

in soil of sixty per cent moisture holding capacity. This was observed in both two times and ten times the normal population (Plates XXII and XXIII). After thirty days, the population remained stable for the duration of the experimental period.

Population Studies of Inoculated Filter Paper

The filter paper was inoculated with B. megaterium and B. cereus to compare population trends of organisms out of their natural environment (Plates XXIV and XXV). The two organisms were the same as those used in soil inoculations. The outstanding difference noted was that the total population was consistently higher than the spore population as compared to the total count to spore count relationship in the soil environment. In addition, the phenomenon of higher spore populations than total populations was not observed in the filter paper environment.

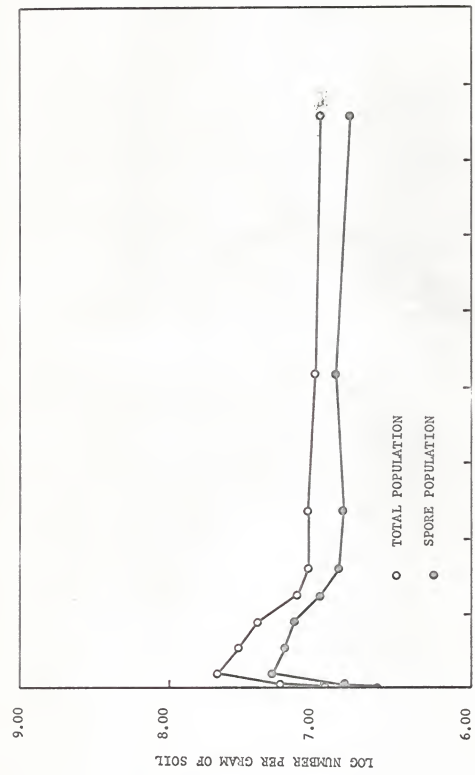
Population Studies of Soils Collected Between 1959 and 1960

These soils were selected for study to support the idea of high sporeforming populations during long periods of drying. The results of this study are shown in Table 3.

EXPLANATION OF PLATE XXII

Bacillus megaterium, at two times the normal population, in soil C at 60% of moisture holding capacity.

PLATE XXII


LOG NUMBER PER GRAM OF SOIL
9.00
8.00
7.00
6.00
20
40
60
80
100
120
140
160
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XXIII

Bacillus megaterium, at ten times the normal population, in soil C at 60% of moisture holding capacity.

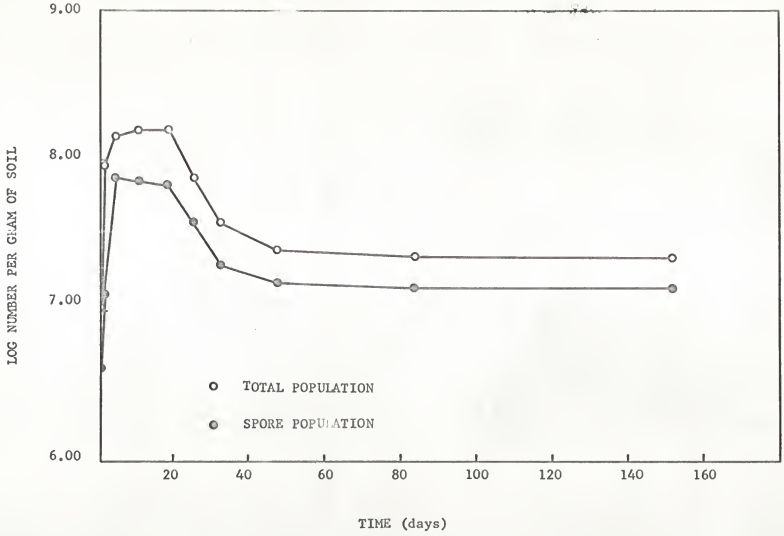
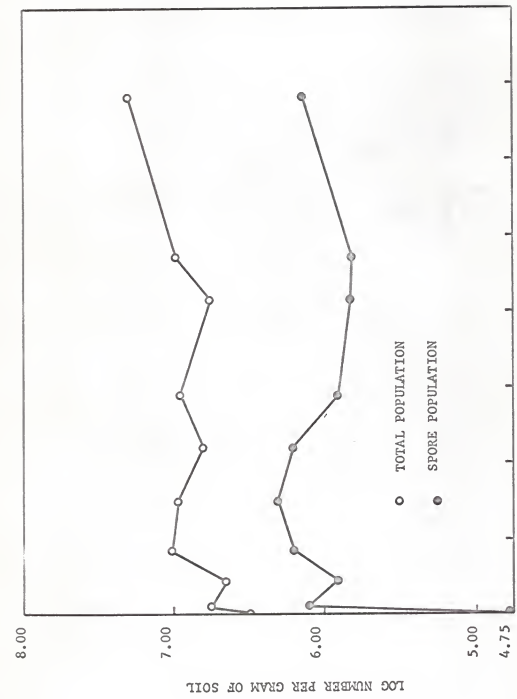

9.00
8.00
7.00
6.00
LOG NUMBER PER GRAM OF SOIL
TOTAL POPULATION
SPORE POPULATION
20
40
60
80
100
120
140
160
TIME (days)

PLATE XXIII

EXPLANATION OF PLATE XXIV

Populations of Bacillus megaterium on dry filter paper.

PLATE XXIV


8.00
7.00
6.00
5.00
4.75
LOG NUMBER PER GRAM OF SOIL
20
40
60
80
100
120
140
TIME (days)
TOTAL POPULATION
SPORE POPULATION

EXPLANATION OF PLATE XXV

Populations of Bacillus cereus on dry filter paper.

PLATE XXV

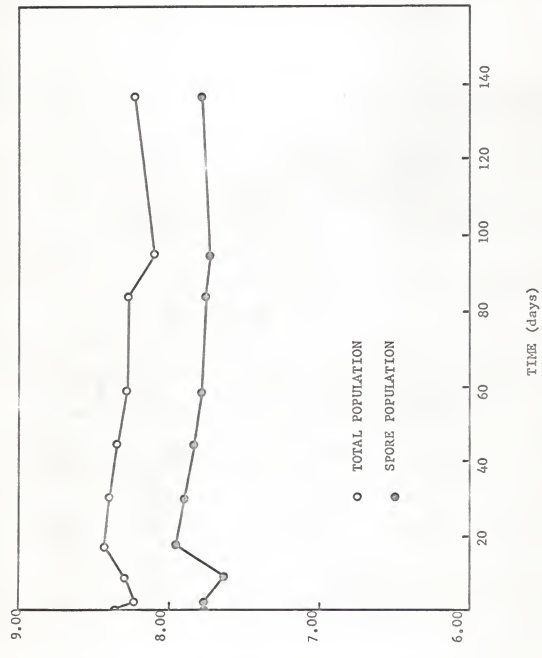

9.00
8.00
7.00
6.00
20
40
60
80
100
120
140
TOTAL POPULATION
SPORE POPULATION
TIME (days)
LOG NUMBER PER GRAM OF SOIL

Table 3. Results of population studies of soils collected between 1959 and 1960.

Soil Number	Original Total Count Per Gm. of Soil	Total Count 1966 Per Gm. of Soil	Spore Count 1966 Per Gm. of Soil
9-61	5.4×10^6	1.25×10^5	1.20×10^5
1-11	2.4×10^6	9.10×10^5	6.90×10^5
4-71	1.4×10^6	1.09×10^6	5.90×10^5
7-31	2.0×10^6	3.20×10^5	2.00×10^5
M-31	7.7×10^6	7.30×10^5	4.00×10^5
K-91	2.2×10^6	1.42×10^5	3.50×10^5
K-46	2.5×10^7	2.04×10^6	1.06×10^6
K-21	1.4×10^6	1.78×10^6	1.04×10^6

Population Survey of Soils Collected in 1926

Only the sandy loam soil showed a population high enough to obtain countable plates. A significant aerobic sporeforming population was not observed in the clay loam soil. The results obtained from the sandy loam soil are as follows:

Total population--2.8×10^5 per gram of soil

Spore population--7.3×10^4 per gram of soil

The results of this survey demonstrated the ability of high aerobic sporeforming populations to survive for extended periods of time in some soils.

DISCUSSION

Murrell (1961) has reviewed the conditions that have an effect on spore formation and germination in a synthetic environment. It was shown that microbial reaction was dependent on a number of physical and chemical factors within the environment, and that any number of changes in these conditions would affect germination and sporulation.

The complex and highly competitive environment of the soil would have an effect on the germination and sporulation of aerobic sporeformers, which in turn would be reflected by population trends. Population numbers, in the four soils previously described, showed more variation in field conditions than in soil under controlled conditions. These results may indicate that each soil sample would represent an individual population and that the population level would be controlled by the environmental conditions in that specific area. The next soil sample would then represent a different population under different environmental conditions. Other factors, such as crop decomposition, competition between organisms, and available nutrients at the time of sampling, may influence population variations.

Decreasing moisture levels and temperatures of 7°C showed a preservative action on aerobic sporeforming populations in soils. The preservation of high sporeforming populations in drying soils was substantiated by the high populations detected in soils that were stored for five years and forty years. Soil type could not be related to population trends. It was noted that high organic soils A, B, and C supported a higher population than the low organic soil D. In most

drying soils and refrigerated soils, the total count was consistently well above the spore count indicating either the existence of vegetative cells or a loss of heat resistance by some spores during pasteurization. In addition, heated spores may have more exacting nutritional requirements than unheated spores (Curran and Evans, 1937).

The inoculated drying soil showed the same population stability. A distinct difference in trends of populations between B. megaterium and B. cereus could not be detected. However, a decline in the total population and the spore population was observed when the two organisms were subjected to a soil of sixty per cent moisture holding capacity. Mahmoud (1957) showed that growth and sporulation of B. subtilis in flooded soil is less than in soil with 12% moisture. He attributed this phenomenon to the dilution of nutrients in flooded soil, to reduced aeration, or to both acting together. It was pointed out by Hardwick and Foster (1952) that sporulation of aerobic sporeforming bacteria could be accelerated by aeration. The decline in population numbers, in soil of sixty per cent moisture holding capacity, may indicate an optimum condition for germination but lack of aeration for sporulation and survival of the vegetative cell which would terminate in non-viable organisms.

B. cereus, in soil of sixty per cent moisture holding capacity, demonstrated several points at which the spore population was actually higher than the total population. Heat activation may explain the occurrence of such a phenomenon due to the pasteurization procedure used in spore enumeration. Heat activation was first explained by

Evans and Curran (1943) and Curran and Evans (1945). They indicated that sublethal heating of spores of many thermo-tolerant and thermophilic aerobes has an influence upon the number of spores which will germinate.

The dry filter paper environment of B. megaterium and B. cereus demonstrated the same population stability as in drying soils. The most significant difference was that the spore population was lower in relation to the total population in the filter paper as compared to the total population to spore population ratio in the soil. This observation may be interpreted as a lack of heat resistance by some spores during pasteurization, or absence of the complex environment of the soil which would induce sporulation.

A population burst of aerobic sporeformers became evident after the addition of peptone. This phenomenon was also demonstrated by Clark and Smith (1949). The question asked here was whether after this burst, would the population remain at the stimulated level during drying, or would the population return to the initial level before the addition of peptone? The results from drying soil indicated that over the period of time studied, the population remained at the stimulated level with little variation in numbers. When dextrose was added twenty days after the peptone addition, a second population burst was not observed. It is generally accepted that fertile soil contains the organic carbon and nitrogen needed for microbial development. However, the absence of another population burst after the addition of dextrose may indicate that the nitrogen level in the soil was not high enough to support a higher population level. It would also indicate that the

aerobic sporeforming population levels are directly related to the carbon-nitrogen ratio in the soil.

SUMMARY

Population variations were more pronounced in soils under field conditions than in soils under controlled conditions. The greatest stability of the aerobic sporeforming population was observed in drying soils. This stability was observed in soils containing the normal aerobic sporeforming flora and soils inoculated with B. megaterium and B. cereus. Soil with high organic matter supported higher populations than the soil of low organic content. Population trends could not be related to soil type. A population decline was noted when the same organisms were subjected to soil of sixty per cent moisture holding capacity.

Populations of B. megaterium and B. cereus were constant on filter paper. The spore population was lower in relation to the total population as compared to the total population to spore population ratio in the soil.

After a population burst, stimulated by the addition of peptone, the population remained at the stimulated level in the drying soil. When dextrose was added twenty days after the peptone addition, a second population burst was not observed.

From the experimental observations, it was suggested that the aerobic sporeforming populations could be affected by such conditions in the soil as high moisture levels, the complex environment, and the carbon-nitrogen ratio.

ACKNOWLEDGEMENT

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APPENDIX

Table 4. Total populations and spore populations of soil A during repeated collections.

Date Collected	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	1.16×10^7	2.20×10^6	26.74%	1.58×10^7	3.00×10^6
6-23-65	1.47×10^7	5.40×10^6	12.12%	1.65×10^7	6.15×10^6
7- 1-65	1.00×10^7	1.81×10^6	25.31%	1.34×10^7	2.42×10^6
7- 8-65	1.03×10^7	2.70×10^6	13.89%	1.20×10^7	3.14×10^6
7-16-65	5.20×10^6	2.50×10^6	12.88%	5.98×10^6	2.87×10^6
7-22-65	2.20×10^6	4.20×10^5	3.52%	2.28×10^6	4.37×10^5
7-29-65	7.70×10^6	8.50×10^5	26.79%	1.05×10^7	1.16×10^6
8-26-65	7.80×10^6	1.52×10^6	18.05%	9.53×10^6	1.86×10^6
9-15-65	1.01×10^7	1.17×10^7	23.20%	1.32×10^7	1.52×10^7
9-30-65	1.16×10^7	4.80×10^6	31.40%	1.69×10^7	7.00×10^6
10-13-65	1.47×10^7	7.60×10^6	13.90%	1.71×10^7	8.81×10^6
11- 9-65	3.80×10^6	2.70×10^6	4.81%	4.00×10^6	2.84×10^6
12- 1-65	9.10×10^6	8.00×10^5	9.79%	1.01×10^7	8.90×10^5
12-30-65	5.40×10^6	1.66×10^6	36.00%	8.50×10^6	2.50×10^6
2- 3-66	1.67×10^6	1.25×10^6	13.00%	1.92×10^6	1.44×10^6

Table 5. Total populations and spore populations of soil B during repeated collections.

Date Collected	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	2.05×10^6	8.30×10^5	20.98%	2.60×10^6	1.05×10^6
6-23-65	2.67×10^6	8.20×10^5	6.53%	2.86×10^6	8.80×10^6
7- 1-65	9.00×10^6	1.90×10^6	32.09%	1.32×10^7	2.80×10^6
7- 8-65	3.60×10^6	8.40×10^5	16.68%	4.34×10^6	1.01×10^6
7-16-65	2.33×10^6	8.60×10^5	4.61%	2.44×10^6	9.00×10^5
7-22-65	1.83×10^6	5.40×10^5	2.13%	1.87×10^6	5.52×10^5
7-29-65	5.90×10^6	7.30×10^5	30.10%	8.45×10^6	1.05×10^6
8- 5-65	5.92×10^6	9.00×10^5	14.00%	6.96×10^6	1.06×10^6
8-26-65	7.10×10^6	2.44×10^6	19.75%	8.86×10^6	3.04×10^6
9-15-65	3.70×10^6	2.10×10^6	26.00%	5.00×10^6	2.84×10^6
9-30-65	6.70×10^6	3.40×10^6	24.70%	8.90×10^6	4.50×10^6
10-13-65	6.40×10^6	1.30×10^6	10.20%	7.12×10^6	1.45×10^6
11- 9-65	7.60×10^6	1.20×10^6	5.95%	8.10×10^6	1.28×10^6
12- 1-65	7.40×10^6	1.30×10^6	9.55%	8.20×10^6	1.44×10^6
2- 3-66	4.90×10^5	6.10×10^5	8.95%	5.40×10^5	6.70×10^5

Table 6. Total populations and spore populations of soil C during repeated collections.

Date Collected	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	7.50×10^6	1.85×10^6	24.70%	9.95×10^6	2.46×10^6
6-23-65	4.30×10^6	1.26×10^6	6.53%	4.52×10^6	1.35×10^6
7- 1-65	9.50×10^6	5.70×10^6	31.75%	1.39×10^7	8.38×10^6
7- 8-65	8.60×10^6	3.20×10^6	15.51%	1.02×10^7	3.80×10^6
7-16-65	4.40×10^6	1.39×10^6	5.38%	4.65×10^7	1.47×10^6
7-22-65	5.00×10^6	1.97×10^6	3.95%	5.20×10^6	2.05×10^6
7-29-65	5.80×10^6	1.90×10^6	28.10%	8.10×10^6	2.75×10^6
8- 5-65	7.00×10^6	1.91×10^6	7.30%	7.50×10^6	2.06×10^6
8-26-65	2.87×10^7	2.94×10^6	17.80%	3.50×10^7	3.58×10^6
9-15-65	6.60×10^6	3.30×10^6	15.00%	7.75×10^6	3.88×10^6
9-30-65	9.70×10^6	4.90×10^6	28.40%	1.35×10^7	6.85×10^6
10-13-65	1.14×10^7	3.30×10^6	12.80%	1.31×10^7	3.79×10^6
11- 9-65	1.23×10^7	4.40×10^6	5.26%	1.30×10^7	4.65×10^6
12- 1-65	6.50×10^6	3.80×10^6	5.60%	6.90×10^6	4.00×10^6
12-30-65	8.50×10^6	2.00×10^6	34.00%	1.29×10^7	3.30×10^6
2- 3-66	3.60×10^6	3.50×10^6	8.35%	3.93×10^6	3.82×10^6

Table 7. Total populations and spore populations of soil D during repeated collections.

Date Collected	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	1.95×10^6	1.22×10^6	8.48%	2.13×10^6	1.33×10^6
6-23-65	3.06×10^6	1.02×10^6	0.63%	3.08×10^6	1.03×10^6
7- 1-65	2.05×10^6	1.02×10^6	8.81%	2.26×10^6	1.12×10^6
7- 8-65	5.90×10^6	1.12×10^6	3.41%	6.10×10^6	1.16×10^6
7-16-65	2.60×10^6	1.85×10^6	0.91%	2.62×10^6	1.87×10^6
7-22-65	3.10×10^6	7.20×10^5	2.46%	3.18×10^6	7.40×10^5
7-29-65	3.08×10^6	6.20×10^5	3.31%	3.18×10^6	6.40×10^5
8- 5-65	3.40×10^6	1.02×10^6	1.11%	3.44×10^6	1.12×10^6
8-26-65	4.40×10^6	1.21×10^6	4.38%	4.60×10^6	1.27×10^6
9-15-65	1.37×10^7	2.50×10^6	14.90%	1.61×10^7	2.94×10^6
9-30-65	1.33×10^7	1.90×10^6	20.20%	1.62×10^7	2.38×10^6
11- 9-65	3.70×10^6	2.30×10^6	6.85%	3.98×10^6	2.48×10^6
12- 1-65	4.90×10^6	3.20×10^6	2.46%	5.00×10^6	3.28×10^6
12-30-65	2.80×10^6	1.07×10^6	19.50%	3.48×10^6	1.33×10^6
2- 3-66	1.40×10^6	1.15×10^6	4.06%	1.46×10^6	1.20×10^6

Table 8. Total populations and spore populations of soil A at 7°C.

Date Sampled	Average Total Count	Average Spore Count
6-17-65	1.16×10^7	2.20×10^6
6-22-65	3.80×10^6	5.80×10^5
6-24-65	1.45×10^6	8.80×10^5
6-28-65	6.90×10^6	4.80×10^6
7- 6-65	1.10×10^7	3.20×10^6
7-14-65	8.10×10^6	5.40×10^6
7-27-65	1.08×10^7	2.90×10^6
8- 3-65	9.70×10^6	2.80×10^6
8-25-65	1.07×10^7	4.30×10^6
10-13-65	1.02×10^7	3.00×10^6
2- 8-66	3.30×10^6	1.12×10^6

Table 9. Total populations and spore populations of soil B at 7°C.

Date Sampled	Average Total Count	Average Spore Count
6-17-65	2.06×10^6	8.30×10^5
6-22-65	1.67×10^6	2.60×10^5
6-24-65	8.70×10^5	6.80×10^4
6-28-65	2.80×10^6	4.80×10^5
7- 6-65	2.86×10^6	1.25×10^6
7-14-65	3.50×10^6	7.10×10^5
7-27-65	2.98×10^6	6.60×10^5
8- 3-65	2.43×10^6	4.10×10^5
8-25-65	3.30×10^6	1.05×10^6
10-13-65	4.80×10^6	1.30×10^6
2- 8-66	1.01×10^6	8.40×10^5

Table 10. Total populations and spore populations of soil C at 7°C.

Date Sampled	Average Total Count	Average Spore Count
6-17-65	7.50×10^6	1.85×10^6
6-22-65	6.60×10^6	1.04×10^6
6-24-65	7.20×10^6	4.90×10^5
6-28-65	6.50×10^6	1.55×10^6
7- 6-65	1.11×10^7	2.80×10^6
7-14-65	1.42×10^7	2.40×10^6
7-27-65	1.64×10^7	2.00×10^6
8- 3-65	5.30×10^6	6.80×10^5
8-25-65	6.20×10^6	3.80×10^6
10-13-65	1.28×10^7	2.90×10^6
2- 8-66	4.20×10^6	2.82×10^6

Table 11. Total populations and spore populations of soil D at 7°C.

Date Sampled	Average Total Count	Average Spore Count
6-17-65	1.95×10^6	1.22×10^6
6-22-65	5.20×10^5	2.10×10^5
6-24-65	1.45×10^6	1.66×10^5
6-28-65	2.20×10^6	6.30×10^5
7- 6-65	6.10×10^6	2.10×10^6
7-14-65	1.36×10^6	8.80×10^5
7-27-65	2.73×10^6	8.50×10^5
8- 3-65	5.30×10^6	6.80×10^5
8-25-65	6.20×10^6	1.14×10^6
10-13-65	9.90×10^6	1.40×10^6
2- 8-66	1.92×10^6	1.08×10^6

Table 12. Total populations and spore populations of soil A at decreasing moisture levels.

Date Sampled	Total Count	Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	1.16×10^7	2.20×10^6	26.74%	1.58×10^7	3.05×10^6
6-22-65	3.58×10^6	7.30×10^5	18.21%	4.38×10^6	8.30×10^5
6-24-65	2.90×10^6	1.02×10^6	15.34%	3.40×10^6	1.20×10^6
6-28-65	4.80×10^6	2.30×10^6	9.17%	5.30×10^6	2.50×10^6
7- 6-65	1.10×10^7	2.80×10^6	3.19%	1.14×10^7	2.89×10^6
7-14-65	8.30×10^6	6.40×10^5	3.62%	8.63×10^6	6.64×10^6
7-27-65	1.00×10^7	2.40×10^6	5.50%	1.06×10^7	2.54×10^6
8- 3-65	3.50×10^6	1.20×10^6	3.35%	3.60×10^6	1.23×10^6
8-25-65	5.00×10^6	2.80×10^6	4.81%	5.26×10^6	2.95×10^6
10-13-65	6.30×10^6	1.30×10^6	2.50%	6.32×10^6	1.32×10^6
2- 8-66	2.21×10^6	1.18×10^6	1.58%	2.23×10^6	1.20×10^6

Table 13. Total populations and spore populations of soil B at decreasing moisture levels.

Date Sampled	Total Count	Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	2.05×10^6	8.30×10^5	20.98%	2.59×10^6	1.05×10^6
6-22-65	3.80×10^6	2.00×10^5	12.76%	4.35×10^6	2.29×10^5
6-24-65	2.40×10^6	1.98×10^5	12.36%	2.74×10^6	2.26×10^5
6-28-65	5.80×10^6	6.90×10^5	6.83%	6.23×10^6	7.40×10^5
7- 6-65	1.49×10^6	9.40×10^5	4.06%	1.55×10^6	9.80×10^5
7-14-65	2.40×10^6	1.36×10^6	3.09%	2.48×10^6	1.40×10^6
7-27-65	2.91×10^6	4.20×10^5	4.72%	3.05×10^6	4.40×10^5
8- 3-65	1.05×10^6	6.00×10^5	4.94%	1.10×10^6	6.30×10^5
8-25-65	2.00×10^6	1.12×10^6	3.75%	2.07×10^6	1.16×10^6
10-13-65	3.40×10^6	1.10×10^6	3.50%	3.45×10^6	1.15×10^6
2- 8-66	1.29×10^6	9.20×10^5	3.20%	1.33×10^6	9.50×10^5

Table 14. Total populations and spore populations of soil C at decreasing moisture levels.

Date Sampled	Total Count	Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	7.50×10^6	1.85×10^6	24.70%	9.93×10^6	2.45×10^6
6-22-65	1.67×10^7	3.80×10^5	14.15%	1.94×10^7	4.44×10^5
6-24-65	1.70×10^6	5.40×10^5	11.73%	1.92×10^6	6.10×10^5
6-28-65	1.05×10^7	1.70×10^6	9.76%	1.16×10^7	1.87×10^6
7- 6-65	9.20×10^6	5.70×10^5	3.41%	9.54×10^6	5.90×10^5
7-14-65	3.30×10^6	1.03×10^6	2.67%	3.38×10^6	1.05×10^6
7-27-65	8.90×10^6	1.85×10^6	4.60%	9.34×10^6	1.94×10^6
8- 3-65	5.40×10^6	7.70×10^5	2.35%	5.53×10^6	7.90×10^5
8-25-65	5.00×10^6	2.12×10^6	3.84%	5.20×10^6	2.20×10^6
10-13-65	9.40×10^6	2.60×10^6	2.00%	9.45×10^6	2.65×10^6
2- 8-66	6.50×10^6	2.17×10^6	1.01%	6.55×10^6	2.22×10^6

Table 15. Total populations and spore populations of soil D at decreasing moisture levels.

Date Sampled	Total Count	Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
6-17-65	1.95×10^6	1.22×10^6	8.48%	2.13×10^6	1.33×10^6
6-22-65	3.50×10^6	4.90×10^5	1.11%	3.52×10^6	4.96×10^5
6-24-65	1.80×10^6	3.30×10^5	1.30%	1.82×10^6	3.34×10^5
6-28-65	3.60×10^6	1.10×10^6	1.32%	3.65×10^6	1.11×10^6
7- 6-65	2.70×10^6	3.20×10^5	0.20%	2.70×10^6	3.20×10^5
7-14-65	1.18×10^6	3.60×10^5	0.20%	1.18×10^6	3.60×10^5
7-27-65	3.02×10^6	1.06×10^6	3.84%	3.15×10^6	1.10×10^6
8- 3-65	2.70×10^6	1.19×10^6	0.64%	2.72×10^6	1.20×10^6
8-25-65	2.90×10^6	1.16×10^6	0.31%	2.92×10^6	1.17×10^6
10-13-65	3.00×10^6	1.60×10^6	0.00%	3.00×10^6	1.60×10^6
2- 8-66	2.31×10^6	1.05×10^6	0.00%	2.31×10^6	1.05×10^6

Table 16. Bacillus cereus, at ten times the normal population, in soil C at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
11-15-65	9.80×10^6	4.30×10^6	14.80%	1.15×10^7	5.10×10^6
11-16-65	1.66×10^8	5.80×10^7	14.90%	1.95×10^8	6.80×10^7
11-19-65	1.11×10^8	3.80×10^7	13.40%	1.29×10^8	4.40×10^7
11-23-65	1.63×10^8	7.80×10^7	8.60%	1.78×10^8	8.50×10^7
11-30-65	1.93×10^8	1.03×10^8	4.50%	2.02×10^8	1.08×10^8
12- 7-65	1.55×10^8	3.90×10^7	3.52%	1.61×10^8	4.05×10^7
12-14-65	3.21×10^8	2.06×10^8	3.52%	3.33×10^8	2.14×10^8
12-21-65	1.61×10^8	7.00×10^7	3.46%	1.67×10^8	7.30×10^7
1- 5-66	1.94×10^8	1.04×10^8	4.10%	2.20×10^8	1.08×10^8
2-10-66	1.58×10^8	7.90×10^7	3.50%	1.63×10^8	8.20×10^7
4-19-66	3.10×10^7	5.50×10^7	2.25%	3.15×10^7	5.55×10^7

Table 17. Bacillus cereus, at two times the normal population, in soil C at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
11-19-65	9.80×10^6	4.30×10^6	14.80%	1.15×10^7	5.10×10^6
11-20-65	5.00×10^7	4.20×10^7	14.80%	5.90×10^7	4.90×10^7
11-23-65	8.20×10^7	9.00×10^7	11.10%	9.20×10^7	1.01×10^8
11-30-65	7.90×10^7	7.80×10^7	11.00%	8.90×10^7	8.80×10^7
12- 7-65	7.30×10^7	5.50×10^7	6.50%	7.80×10^7	5.90×10^7
12-14-65	7.00×10^7	7.90×10^7	5.05%	7.40×10^7	8.30×10^7
12-21-65	6.70×10^7	9.30×10^7	4.50%	7.00×10^7	9.70×10^7
1- 5-66	7.20×10^7	5.80×10^7	3.62%	7.50×10^7	6.00×10^7
2-10-66	3.90×10^7	3.80×10^7	2.20%	4.00×10^7	3.90×10^7
4-19-66	7.50×10^7	4.80×10^7	1.53%	7.52×10^7	4.82×10^7

Table 18. Bacillus megaterium, at ten times the normal population, in soil C at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
11-15-65	9.80×10^6	4.30×10^6	14.80%	1.15×10^7	5.10×10^6
11-16-65	2.56×10^8	6.80×10^7	15.50%	3.30×10^8	8.00×10^7
11-19-65	1.86×10^8	9.80×10^7	13.90%	2.16×10^8	1.13×10^8
11-23-65	2.61×10^8	1.88×10^8	7.80%	2.83×10^8	2.04×10^8
11-30-65	1.35×10^8	7.90×10^7	4.95%	1.42×10^8	8.30×10^7
12- 7-65	1.35×10^8	8.30×10^7	4.50%	1.41×10^8	8.70×10^7
12-14-65	7.90×10^7	6.40×10^7	3.74%	8.20×10^7	6.60×10^7
12-21-65	9.00×10^7	4.80×10^7	3.50%	9.30×10^7	4.90×10^7
1- 5-66	1.28×10^8	7.80×10^7	2.90%	1.32×10^8	8.00×10^7
2-10-66	1.90×10^8	2.32×10^8	1.94%	1.94×10^8	2.36×10^8
4-19-66	5.30×10^7	7.30×10^7	1.50%	5.40×10^7	7.40×10^7

Table 19. Bacillus megaterium, at two times the normal population, in soil C at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
11-19-65	9.80×10^6	4.30×10^6	14.80%	1.15×10^7	5.10×10^6
11-20-65	2.56×10^7	5.50×10^6	15.60%	3.30×10^7	6.50×10^6
11-23-65	5.50×10^7	3.30×10^7	9.05%	6.05×10^7	3.64×10^7
11-30-65	5.40×10^7	2.22×10^7	4.72%	5.80×10^7	2.33×10^7
12- 7-65	2.90×10^7	1.65×10^7	4.05%	3.03×10^7	1.72×10^7
12-14-65	4.10×10^7	3.70×10^7	3.62%	4.25×10^7	3.85×10^7
12-21-65	5.00×10^7	3.80×10^7	3.38%	5.20×10^7	3.94×10^7
1- 5-66	5.50×10^7	3.80×10^7	2.56%	5.60×10^7	3.80×10^7
2-10-66	5.10×10^7	4.90×10^7	1.84%	5.20×10^7	5.00×10^7
4-19-66	3.10×10^7	2.80×10^7	1.63%	3.20×10^7	2.90×10^7

Table 20. Bacillus cereus, at ten times the normal population, in soil C at 60% of moisture holding capacity.

Date Sampled	Average Total Count	Average Spore Count
11-19-65	9.80×10^6	4.30×10^6
11-20-65	1.37×10^8	1.36×10^8
11-23-65	1.21×10^8	1.25×10^8
11-30-65	1.92×10^8	1.70×10^8
12- 7-65	1.28×10^8	1.47×10^8
12-14-65	1.12×10^8	1.04×10^8
12-21-65	6.80×10^7	8.80×10^7
1- 5-66	6.90×10^7	4.50×10^7
2-10-66	4.00×10^7	5.90×10^7
4-19-66	3.30×10^7	3.80×10^7

Table 21. Bacillus cereus, at two times the normal population, in soil C at 60% of moisture holding capacity.

Date Sampled	Average Total Count	Average Spore Count
11-19-65	9.80×10^6	4.30×10^6
11-20-65	3.90×10^7	4.10×10^7
11-23-65	3.20×10^7	3.30×10^7
11-30-65	3.40×10^7	3.80×10^7
12- 7-65	5.70×10^7	5.90×10^7
12-14-65	4.70×10^7	3.10×10^7
12-21-65	2.08×10^7	2.32×10^7
1- 5-66	1.64×10^7	1.42×10^7
2-10-66	1.42×10^7	1.54×10^7
4-19-66	1.24×10^7	1.31×10^7

Table 22. Bacillus megaterium, at ten times the normal population, in soil C at 60% of moisture holding capacity.

Date Sampled	Average Total Count	Average Spore Count
11-19-65	9.80×10^6	4.30×10^6
11-20-65	9.40×10^7	1.32×10^7
11-23-65	1.43×10^8	7.80×10^7
11-30-65	1.57×10^8	7.30×10^7
12- 7-65	1.67×10^8	7.00×10^7
12-14-65	7.50×10^7	3.80×10^7
12-21-65	3.80×10^7	2.01×10^7
1- 5-66	2.60×10^7	1.54×10^7
2-10-66	2.28×10^7	1.44×10^7
4-19-66	2.27×10^7	1.40×10^7

Table 23. Bacillus megaterium, at two times the normal population, in soil C at 60% of moisture holding capacity.

Date Sampled	Average Total Count	Average Spore Count
11-19-65	9.80×10^6	4.30×10^6
11-20-65	1.83×10^7	6.90×10^6
11-23-65	4.90×10^7	2.08×10^7
11-30-65	3.50×10^7	1.73×10^7
12- 7-65	2.64×10^7	1.49×10^7
12-14-65	1.46×10^7	1.03×10^7
12-21-65	1.20×10^7	7.40×10^6
1- 5-66	1.24×10^7	7.00×10^6
2-10-66	1.10×10^7	8.00×10^6
4-19-66	1.00×10^7	6.40×10^6

Table 24. Populations of Bacillus megaterium on dry filter paper.

Date Sampled	Average Total Count	Average Spore Count
12- 4-65	3.20×10^6	5.70×10^4
12- 6-65	5.60×10^6	1.32×10^6
12-13-65	4.50×10^6	8.00×10^5
12-21-65	1.07×10^7	1.58×10^6
1- 3-66	9.40×10^6	1.98×10^6
1-17-66	6.30×10^6	1.55×10^6
1-31-66	9.20×10^6	7.90×10^5
2-25-66	5.80×10^6	6.60×10^5
3- 8-66	9.60×10^6	6.30×10^5
4-19-66	1.97×10^7	1.22×10^6

Table 25. Populations of Bacillus cereus on dry filter paper.

Date Sampled	Average Total Count	Average Spore Count
12- 4-65	2.30×10^8	5.90×10^7
12- 6-65	1.69×10^8	5.80×10^7
12-13-65	2.01×10^8	4.30×10^7
12-21-65	2.68×10^8	9.40×10^7
1- 3-66	2.51×10^8	7.90×10^7
1-17-66	2.25×10^8	6.90×10^7
1-31-66	1.92×10^8	6.20×10^7
2-25-66	1.87×10^8	5.80×10^7
3- 8-66	1.35×10^8	5.40×10^7
4-19-66	1.68×10^8	6.10×10^7

Table 26. Total populations and spore populations of soil C, with peptone and dextrose additions, at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
7- 8-65	8.60×10^6	3.20×10^6	15.51%	1.02×10^7	3.80×10^6
7-13-65	2.69×10^8	1.01×10^8	14.81%	3.16×10^8	1.19×10^8
7-19-65	2.72×10^8	1.03×10^8	8.81%	2.98×10^8	1.13×10^8
7-23-65	1.61×10^8	7.10×10^7	1.04%	1.63×10^8	7.20×10^7
7-28-65	1.89×10^8	8.80×10^7	5.50%	2.00×10^8	9.30×10^7
8- 2-65	2.71×10^8	9.80×10^7	24.65%	3.60×10^8	1.30×10^8
8-24-65	1.19×10^8	1.04×10^8	6.95%	1.28×10^8	1.12×10^8
10-12-65	1.22×10^8	4.90×10^7	2.20%	1.25×10^8	5.00×10^7
4-19-66	6.30×10^7	3.60×10^7	1.23%	6.40×10^7	3.70×10^7

Table 27. Total populations and spore populations of soil C, with the addition of peptone, at decreasing moisture levels.

Date Sampled	Average Total Count	Average Spore Count	Moisture	Corrected Total Count	Corrected Spore Count
7- 8-65	8.60×10^6	3.20×10^6	15.51%	1.02×10^7	3.80×10^6
7-13-65	2.69×10^8	1.01×10^8	14.81%	3.16×10^8	1.19×10^8
7-19-65	2.72×10^8	1.03×10^8	8.81%	2.98×10^8	1.13×10^8
7-23-65	1.61×10^8	7.10×10^7	1.04%	1.63×10^8	7.20×10^8
7-28-65	1.89×10^8	8.80×10^7	5.50%	2.00×10^8	9.30×10^7
8- 2-65	1.68×10^8	7.40×10^7	4.38%	1.76×10^8	7.75×10^7
8-24-65	1.81×10^8	9.40×10^7	6.95%	1.94×10^8	1.01×10^8
9- 8-65	1.15×10^8	8.90×10^7	3.20%	1.19×10^8	9.20×10^7
10-12-65	1.58×10^8	7.50×10^7	1.73%	1.61×10^8	7.60×10^7
11-11-65	1.26×10^8	5.60×10^7	2.88%	1.30×10^8	5.80×10^7
12- 8-65	1.27×10^8	8.00×10^7	3.20%	1.31×10^8	8.30×10^7
2- 1-66	4.80×10^7	5.70×10^7	2.36%	4.90×10^7	5.85×10^7

Table 28. Total populations and spore populations of soil C, with the addition of peptone, in a plastic bag to retain the initial moisture level.

Date Sampled	Average Total Count	Average Spore Count
7- 8-65	8.60×10^6	3.20×10^6
7-19-65	2.73×10^8	7.10×10^7
7-23-65	2.02×10^8	6.30×10^7
7-28-65	2.47×10^8	8.50×10^7
8- 2-65	1.67×10^8	1.05×10^8
8-24-65	1.47×10^8	9.30×10^7
9- 8-65	9.90×10^7	8.00×10^7
10-12-65	1.02×10^8	6.00×10^7
11-11-65	6.60×10^7	5.30×10^7
12- 8-65	8.50×10^7	7.00×10^7
2- 1-66	5.20×10^7	4.80×10^7

AN INVESTIGATION OF POPULATIONS OF AEROBIC SPOREFORMING BACTERIA IN THE SOIL

by

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A population study was made on viable spores of aerobic spore-forming bacteria compared with total numbers growing on nutrient agar. Four Kansas soils were analyzed under different conditions of moisture, temperature, and available nutrients. Population trends were followed for seven months. It was shown that variations in population numbers were more pronounced in soils under field conditions than in soils under controlled conditions.

Soils with decreasing moisture levels and soils held at 7°C showed a preservative action on the aerobic sporeforming populations. Also, high sporeforming populations were demonstrated in soils that were stored for five years and for forty years.

Populations of Bacillus megaterium and Bacillus cereus, that were inoculated into a soil containing 2.6% organic matter, demonstrated population stability in a soil at decreasing moisture levels. Declines in the total population and spore population were observed when the two organisms were subjected to a soil held at 60% moisture holding capacity.

Filter paper was inoculated with B. megaterium and B. cereus to compare population trends in cultures removed from their natural environment. The dry filter paper environment demonstrated the same population stability as the drying soils. The most significant difference was that the spore population was lower in relation to the total population as compared to the total population to spore population ratio in the soil.

When peptone was added to a soil high in organic matter (2.6%) the population of aerobic sporeformers increased considerably. This population burst took place at decreasing moisture levels. During the

experiment, the population remained stable at the stimulated level. No second burst was observed when dextrose was added 20 days after the peptone enrichment. The absence of such a second population burst may indicate that the nitrogen level in the soil was not sufficiently high enough to support a higher population. This implies that the population levels of aerobic sporeforming bacteria are directly related to the carbon-nitrogen ratio in the soil.