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The Occurrence of Antibiotic Actinomyces in the Sugarcane Soils of Louisiana and Their Effect Upon Pythium Root Rot.

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THE OCCURRENCE OF ANTIBIOTIC ACTINOMYCES IN THE SUGARCANE SOILS OF LOUISIANA AND THEIR EFFECT UPON PYTHIUM ROOT ROT

A Dissertation

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ABSTRACT

The non-pathogenic microflora has been considered as a possible ecological factor in the etiology of root rot of sugarcane. These studies dealt with the group, Actinomyces. These organisms are abundant in most soils and are known to include forms which are antibiotic to Pythium arrhenomanes, the causative agent of sugarcane root rot. This pathogen occurs in most Louisiana sugarcane soils, although serious outbreaks of the disease are sporadic. Nutritional and environmental factors have been shown to be responsible for some of this variation. However, these factors are not always sufficient to account for the differences in the severity of the root rot disease.

The Actinomyces populations of soil samples collected throughout the cane belt were determined by means of a standard dilution and culture procedure. The antibiotic activity of individual Actinomyces isolates against Pythium arrhenomanes was determined for a number of isolates from each soil sample.

The Actinomyces populations were not correlated with the soil pH, seasons, or the soil type, but they were affected by the amount of rainfall. The percentage of antibiotic isolates varied from 18.51 to 31.46 for the different soil types. In general there was close agreement between the percentage of antibiotic cultures and the antibiotic index; that is, the average distance of inhibition in millimeters. The antibiotic index was not influenced by the rainfall, but did show a positive correlation with the soil reaction and varied widely in the different soils.

The soils of the sugarcane area were grouped into five different types. The average yield of sugarcane for each of these soil types was determined. These values were based upon the yields of three varieties grown for a number of years in several test plots in each soil type. The correlation between the average sugarcane yield and the antibiotic index was very close. The product of the antibiotic index and the number of thousands of Actinomyces per gram soil, which was termed the antibiotic value of the Actinomyces population, was even more closely correlated with the average yields. The close correlation as shown by this study suggests that the antibiotic Actinomyces may influence the yielding capacity of the soil, perhaps by limiting the virulence of the pathogenic Pythium.

There were from 2.8 to 33.4 times as many Actinomyces in the immediate vicinity of sugarcane roots as in the root-free soil. The antibiotic index was approximately the same for the Actinomyces from the two regions. This fact is considered of importance, since the total antibiotic activity of the Actinomyces would be greater in the immediate vicinity of the roots.

Various amendments were found to influence the Actinomyces population when added to unsterilized soil, either under field or laboratory conditions. The antibiotic index was less sensitive to these changes, but was influenced by some of the amendments and by the pH of the soil. These facts suggest means of changing the antibiotic microflora of the soil.

Greenhouse studies demonstrated that certain of the antibiotic Actinomyces increased rapidly when introduced into sterilized soil. Some of the isolates decreased the severity of root rot of corn plants

in greenhouse tests. The degree of control was greater several weeks after Pythium and Actinomyces had been added to the soil.

Limited isolations made from field soils to which an antibiotic Actinomyces had been added failed to show any increase of the introduced isolate.

INTRODUCTION

The variability of the severity of sugarcane root rot, caused by Pythium arrhenomanes Drech., has not been satisfactorily explained upon the basis of distribution of the pathogen, soil moisture, soil temperature, soil types, nutritional factors, or upon the occurrence of soil toxins. The effect of the non-pathogenic microorganisms upon this disease has received little attention. Tims (106) reported the occurrence of Actinomyces, antibiotic to Pythium arrhenomanes, in Louisiana sugarcane soils. LeBeau (69) found antibiotic Trichoderma isolates to be quite common in the cane soils.

The inhibition of one microorganism by another has received much attention during the last decade. However, most of the studies have been limited to the laboratory or greenhouse. The part that these antibiotic organisms play in the field soils in holding the soil-borne organisms to a given level is still uncertain. Among the antibiotic organisms in the soil, Actinomyces are perhaps the most abundant, and they are readily isolated and cultured in the laboratory.

The present study was initiated to determine the distribution of the antibiotic organisms in Louisiana cane soils and to study the possibility of utilizing them to reduce the root rot disease, either by inoculation into the soil or by application of cultural practices to favor the development of the antibiotic strains.

HISTORICAL

Sugarcane Root Rot

Root rot of sugarcane was first described in Java by Wakker in 1885. He described Marasmius saccharii Wak. as the causative agent. Many of the earlier workers who studied root failure in the different countries also considered this or related species to be the pathogens. Among those following this concept, at least for a time, were Howard in the West Indies in 1903 (60), Lewton-Brain (72), and Cobb (21) in Hawaii, Fulton (46) and Edgerton (32) in Louisiana. However, it should be pointed out that Cobb considered Ithyphallus coralloides Cobb to be the main cause.

In 1919, Lyon (76) disproved the pathogenicity of these two fungi and reported the occurrence of Chytrid-like bodies in the diseased roots and Carpenter (7) demonstrated that a Pythium caused typical symptoms when inoculated into sterilized soil. Further evidence of its pathogenicity was reported in 1920 (8). He also discussed the effect of temperature upon the severity of the disease.

Earle and Matz in 1920 (30) in Porto Rico considered Rhizoctonia and Pythium to be the causal agents. Bourne, two years later (See 77), reported experiments showing that Rhizoctonia solani Kuhn and R. palidia Matz were pathogenic on cane roots. Edgerton and others in Louisiana in 1924 (34) gave experimental evidence showing that species of Pythium, in addition to Marasmius, were important in causing root rot. Later workers (35, 87) indicated that Rhizoctonia may produce severe disease symptoms on the old noble canes under certain conditions.

Several investigators have considered the injury caused by various soil animals. As early as 1909, Cobb (22) had observed that two nematodes, Heterodera radicola Mull. and Tylenchus biformis Cobb, were attacking sugarcane roots, and suggested that they have a part in root failure. Muir and Henderson (83) suggested that nematode injury might be two-fold; direct, and secondarily through the predisposition of cane roots to fungus attacks through the lesions. Rands in Louisiana (86), Van Zwaluwenburg in Hawaii (111), and Spencer and Stracener in Louisiana (99, 100) extended the list of insects attacking sugarcane roots to include other nematodes, snails, springtails, centipedes, earth worms and small beetles.

More recent studies, especially in Louisiana (34, 36) and in Hawaii, numerous reports which were summarized by Carpenter (11) have shown that Pythium arrhenomanes is the principle causative agent of sugarcane root rot.

The pathogen was first identified by Carpenter (9) as Pythium butleri Subr. In 1928 (10), after reconsidering its taxonomy, he identified it with P. aphanidermatum (Edson) Fitzpatrick. And still more recently, following taxonomic comparisons, Rands and Dopp (89) changed it to P. graminicolum Subr. However, Carpenter (14) considered P. graminicolum as synonymous with P. arrhenomanes Dresch., with the latter having priority. Dreschler (29) distinguished between the two species, and identified the sugarcane pathogen as P. arrhenomanes Dresch.

Since the demonstration of the pathogenicity of Pythium, much work has centered around it and the effect of soil conditions upon the severity of the disease. Hawaii has led in these studies. Carpenter (10), considering the accumulated observations made over a period of

years, developed the working hypothesis that nutritional factors were the most important part of the root disease complex. In a later series of studies (11, 12) he demonstrated that the addition of nitrogenous substances often resulted in severe root rot in soils in which the disease had never been a serious problem. Rands (88) reported considerable control of the disease in heavy soils of Louisiana by application of filter press mud. Further evidence of this was later presented (90). Later, Rands and Dopp (91) observed that root rot injury was less severe in heavy soils which had had all cane trash turned under over a period of years, than in untreated areas.

In Canada, browning root rot of wheat, caused by Pythium arrhenomanes var. canadensis Van. and Trus., was also found to be more severe following the application of nitrogenous fertilizers and greatly reduced following the application of phosphatic fertilizers (109, 110). Cooke (24) obtained a remarkable decrease of root rot by heavy application of phosphates to certain soils. This was confirmed and enlarged upon by Carpenter (14), working with inoculated sterilized soil. Also, by an isolated-root nutrition technique, he (15) demonstrated that a single root growing in phosphate solution could adsorb enough phosphorous to prevent development of root rot on the remainder of the root system.

McGeorge (78) found the "Lahaina disease" to be more severe on acid soils, but considered it was due to the toxicity of certain soluble salts and not to acidity per se. Lee and others (70) reported that the occurrence of Pythiums was correlated to slightly alkaline soils and that a contributing factor to "Lahaina disease" was puddling. The early Java investigators had considered this as a cause of root failure

(see 76). O'Neal and Schreiner (84) found a rough correlation between these factors, but under Louisiana conditions this was usually overshadowed by the effect of drainage. Flor (44, 45) reported growth of a pathogenic isolate of Pythium between pH 5.3 - 9.2.

Mineral toxicity has received considerable attention by Hawaiian workers. Even after they had proven the pathogenicity of Pythium, Lyon (76) and Carpenter (7) recognized the importance of the toxicity of certain elements in the soil solution. The work of McGeorge (78) on this phase of the problem is outstanding. He demonstrated the toxicity of aluminum salts in certain Hawaiian soils. He did not consider this as the cause of the "Lahaina disease" but rather as one of the factors causing low fertility of these soils. Carpenter (14) suggests that excess of calcium, magnesium, and sodium may also be predisposing factors of root rot. As has already been pointed out, this is also true for nitrogen. However, on Florida peat soils this effect of nitrogen is overcome by the addition of the minor elements, copper and zinc (91).

Summarizing the effects of unbalanced nutrients upon the predisposition of sugarcane to infection by Pythium, Carpenter (14) concluded that resistance or susceptibility of a given variety was largely determined by its ability to tolerate these unbalanced nutritive conditions.

The accumulation of organic toxins in poorly drained soils has been considered as a predisposing factor. Tyron in 1905 (108) first mentioned this as a possible explanation of the injurious effect of organic material. Flor (44, 45) failed to find evidence of such toxins in seepage soils along the Mississippi River. Rands and Dopp (90) studied the effect of sub-toxic amounts of hydrogen sulfide and

salicylic aldehyde in greenhouse sand-nutrient culture. Both of these are normal by-products of anaerobic decomposition. The salicylic aldehyde apparently disposed the cane to infection by a weakly parasitic Pythium isolate, so much so that growth reduction was from two to seven times as great as with Pythium alone. Thus if certain toxic substances should accumulate they might be of importance in the predisposition of cane to root rot.

The first suggestion that temperature was important in determining the severity of the disease was made by Carpenter in 1920 (8). This observation was again made by Edgerton and others (33, 35, 36) in Louisiana where cane remains dormant for three to four months of the winter period. Cool, wet spring weather which retards growth resulted in severe root rot. Flor (44, 45) confirmed these observations by growing plants under a range of controlled temperature and soil moisture conditions. This was reconfirmed by Bands and Dopp (91). Similar observations have been made concerning the effect of temperature upon Pythium root rot of corn (62).

This brief review of the literature shows the complexity of the root rot disease of sugarcane, as it is influenced by different nutritional ratios and by climatic conditions. Yet, these hardly account for the variations of the severity of a disease which is caused by such a widespread pathogen. One other factor that could possibly influence the disease expression is the effect of the saprophytic soil microflora.

Antagonistic Organisms in the Soil

Soil-borne plant parasites are directly involved in two biological relationships; first, with the host, and second, with the entire soil microflora surrounding it, i.e., the ecological position of the pathogen.

The development of any introduced pathogen in the soil has to meet continued competition with the remainder of the microorganisms. This phase of antagonism has proven to be important in the control of certain diseases, especially those caused by pathogens not well adapted to a saprophytic mode of livelihood (53). However, the antagonism of soil-borne pathogens by specific soil inhabitants which produce specific toxins has also been repeatedly demonstrated. Both types of antagonism have received considerable attention during the last two decades, as evidenced by numerous reviews (47, 50, 52, 53, 112, 113, 114, 122).

Take-all of wheat has been the object of much research along this line of inquiry. Sanford and Broadfoot (95) tested both living cultures and filtrates of 23 fungi, 3 Actinomyces, and 40 bacteria, and found that 11 of the fungi, 2 of the Actinomyces, and 23 of the bacteria exerted at least some control effect. In general the bacteria were much less active. Wheat plants grown in sterile and unsterile soil inoculated with Ophiobolus graminis Sacc. showed no difference in the severity of the disease at 13°C., but it was much more severe in the

sterilised soil when grown at 27°C. (58). This difference in virulence was interpreted as a temperature effect upon the saprophytic microflora.

Broadfoot (5) found O. graminis to be more virulent when inoculated alone than with either of three other pathogens: Fusarium culmorum (W. G. Sm.) Sacc., Helminthosperium sativum P. K. & B., or Leptosphaeria lepo-trichoides de Nat.. Garrett, after a series of analytical studies (49, 50, 51), concluded that competition of the microbial population affected the survival of O. graminis. Following this, Stumbo and others (103) found that available nitrogen and phosphate decreased infection, even though no marked change occurred in the microbial numbers; neither were they able to detect qualitative or quantitative differences in the microflora, or in available nutrients, in infested and non-infested areas of field soils.

According to Fellows and Ficke (43) the pathogen failed to spread from infested soil to non-infested soil unless wheat roots extended across the boundary. O. graminis injury was much less severe when inoculated into recontaminated sterilized soil than in unsterilized infested soil (75). This was considered due to differences in growth rate of pathogen and the saprophytic microflora. Slagg and Fellows (98) studied organisms for their inhibition of O. graminis in infested and inoculated soil. Several of the Moniliales were active in this respect.

Foot-rot of wheat is caused by Fusarium culmorum and Helminthosperium sativum. These pathogens are more persistent in the soil than O. graminis, and are considered to be better adapted to a saprophytic existence. Porter in 1924 (85), one of the earlier students of interaction between microorganisms, placed a layer of soil containing the saprophyte over one containing H. sativum in large test tubes, then

grew a wheat seedling in this. He obtained a "measurable" control of root rot on wheat seedlings. The same procedure gave similar results with flax wilt.

Henry (57) greatly reduced the development and sporulation of F. culmorum and H. sativum by adding a small portion of unsterile soil or certain saprophytes to sterilized soil. According to Bisby and others (4), one per cent of the fungal isolates from certain Canadian soils was Trichoderma lignorum (Tode) Harz. This fungus greatly reduced the virulence of foot-rot pathogens in sterilized soil. Greaney and Machacek (54) obtained similar results with Trichothecium roseum Cda. against H. sativum. The list of organisms antagonistic to these pathogens in the soil was further extended to include isolates of Penicillium and Actinomyces by Sanford and Cormack (96). They concluded that many isolates of genera or species may differ widely in antagonistic activity.

Fusarium oxysporium Schlect. var. cubense (E. F. Sm.) Woll. and Rein., causative agent of banana wilt, was found to be inhibited by about 12% of the Actinomyces isolated from Jamaican soils (79). There was no appreciable control when infested field soil was inoculated with these antibiotic isolates.

Phymatotrichum omnivorum Shear, causative agent of cotton root rot, with a wide host range and a sclerotial stage, is able to survive in the soil for long periods. In the irrigated region of Arizona, King and Loomis (68) obtained control by burying heavy applications of organic matter in the fall and flooding immediately. They later confirmed the hypothesis that control was due to increased microbiological activity, as shown by increased respiration and increased numbers of

organisms on modified Cholodny slides (17) in the treated plots (66, 67). Rhea (92) failed to get similar control in the unirrigated black lands of Texas.

Trichoderma lignorum was reported as toxic to P. omnivorum (6) and to reduce severity of the disease when heavily inoculated into infested soil (105). Mitchell and others (82) found the pathogen to be more sensitive to microbial activity in the absence of living host tissue.

Ezekiel and others (42) isolated a toxic substance from monocot roots and attributed their resistance to P. omnivorum to this fact. However, Eaton and Rigler (29) later presented evidence to show that the microflora associated with corn roots was responsible for resistance.

Rhizoctonia solani Kuhn causes stem canker of potatoes, damping-off of numerous seedlings and other root diseases. As early as 1918, Hartley and others (56) observed that certain saprophytic fungi inoculated into steam-sterilized soil along with Corticium vagum B. and C. or Pythium debaryanum Hesse reduced the severity of the disease on forest seedlings. Weindling (119) reported that Trichoderma lignorum parasitized and inhibited R. solani. In further studies (120, 121) he compared the action of T. lignorum and Gliocladium. T. album Preuss and T. koningi Oud. also attacked R. solani.

Allen and Hasnseler (2) reduced Rhizoctonia infection of peas and cucumbers from 33 and 37% to 28 and 12% respectively, by adding T. lignorum to heavily-infested soil. Weindling and Fawcett (123) successfully controlled Rhizoctonia damping-off of citrus seedlings by adding T. lignorum to acidified soil or to peat moss with which the seed was covered. Daines (28) reduced the amount of stem canker of

potato developing from infected tubers by dipping them in a spore suspension of T. lignorum before planting or by applying the suspension about the base of the young plants. Cordon and Haenseler (27) isolated a rough strain of Bacillus simplex Gottheil which produced a heat-stable toxin, that inhibited R. solani in culture and soil. Washed bacterial cells added to the soil also gave appreciable control of cucumber damping-off. Sanford (94) found R. solani to be more virulent on potato stems in unsterilized soil, although mycelial and sclerotial development were greater on sterilized soil.

Endo reported (37, 38, 39, 40) studies of the inhibition of three pathogens (Hypochnus centrifugus (Lev.) Tul., H. sasakii Shirai, and Sclerotium oryzae-sativae Sawada) by stock cultures of Bacilli, Bacteria, Aspergilli, (several cultures of A. niger v. Tieg.), Penicilli, Mucors, and a single Absidia. In general, H. centrifugus was more resistant to antibiotic activity, especially that of the fungi, than the other two pathogens. Inhibition in the soil, as shown by infection of respective hosts, was lower than in culture. However, the same general trends were evident.

Actinomyces scabies (Thaxt.) Gussow reportedly survives almost indefinitely in soil under field conditions. Millard and Taylor (81) reported control of scab by heavily inoculating infested soil with a saprophytic Actinomyces, A. praecox Mill. and Burr. The degree of control was correlated with the amount of inoculum added; thus they considered control was due to competition. Sanford (93) failed to confirm these findings in later studies.

Strawberry root rot is one of the so-called disease complexes. Several organisms have been isolated and their pathogenicity proven. Hildebrand and West (59), studying the effect of a succession of green

manurial treatments on the disease, used the following treatments: 1) steam sterilized annually, 2) soybeans, 3) barnyard manure applied annually, 4) corn, 5) red clover, 6) timothy, and 7) untreated. Two to three crops of the cover-crop treatments were grown annually. Young strawberry plants were transplanted to the soil once each year. The severity of the disease was in the order listed. Classification of the bacteria isolated from the treated soil on the basis of nutritive requirements showed a selective action of the treatments for certain groups of bacteria which were very closely correlated with disease severity. Further study (West and Hildebrand, 117) showed that selectivity was not due to the growing cover crops but to their decomposition, i.e., the decomposition of soybeans reduced the bacteria associated with root rot. Gries (55) found that a substance toxic to strawberries was synthesized by the bacteria associated with the red clover plants. Thus, sterile soybean tissue decomposed by the bacteria associated with the red clover plants produced the toxin, and sterile red clover plants decomposed by bacteria from soybean plants was non-toxic. Recently, Cochrane (23) has shown the toxin to be present in undecomposed clover and rye grass tissue. However, these and toxins produced during decay of certain other plant material were destroyed by further microbial activity.

Sugarcane root-rot caused by Pythium arrhenomanes Drech. (7) is dependent upon several factors: nutritive level and ratios (13), soil temperature and moisture (45) and varietal resistance. The effect of the microflora on the diseases has received considerable attention. Tims (106) isolated an Actinomyces that was antagonistic to the Pythium in culture and greatly reduced the severity of the disease in pot culture. Rands (88) reported considerable control of root rot under

field conditions by using filter press mud. He attributed this control to increased microbial activity. Rands and Dopp (89) gave still further evidence of such control. They presented an extensive review of the literature concerning this disease up to 1938 (91).

LeBeau (69) found that one per cent of the Trichoderma isolates from cane soils were antagonistic to P. arrhenomanes. He obtained a fair degree of root rot control in greenhouse culture using corn plants and increased cane yields in the field by inoculating the soil with Trichoderma. Allen and Haenseler (2) had previously reported that a Trichoderma inhibited Pythium debaryanum Hesse in culture and in the soil. Later Anslow et. al., 1943 (3), demonstrated that four species of Pythium were inhibited by an antibiotic produced by two species of Penicillium. Meredith and Semeniuk (80), isolating from two Iowa soils, found about 21% of the isolates to be antibiotic to P. graminicolum Subr. The isolate that they inoculated into the soil gave some control if the broth containing the Actinomyces was added to the infected soil, but not when Actinomyces spores alone were added to the soil.

Antagonistic organisms are common in the three groups, Bacteria, Fungi, and Actinomyces. The percentage of active organisms varies widely in different genera of these groups. Actinomyces were chosen for this survey since they are widely distributed and preliminary studies indicated them to be numerous, with a fairly high percentage antibiotic.

MATERIALS AND METHODS

Soil samples were collected from several different plantations in each of the four major sugarcane areas of Louisiana. Samples were taken from both light and heavy soils in the alluvial area along the Mississippi River and Bayou Lafourche. Collections of samples were made at intervals over a 20 month period; that is, through two growing seasons. Five to ten pounds of soil were taken in each sample. The samples of soil were stored in paper bags under laboratory conditions until the dilutions could be made. This was usually within less than a week after collection.

For isolation, a representative 100 gram sample, passed through a 4-mesh soil screen, was placed in a liter of water drawn from the hot water tap and cooled to room temperatures. These were periodically shaken, with each sample receiving the same amount of agitation. After approximately three hours, 1 cc. of the suspension was withdrawn in a sterile pipette just following vigorous agitation. This aliquot was added to 100 cc. of sterilized water in a flask plugged with cotton. The flask was then shaken and a 1 cc. portion of it transferred with a second sterile pipette to another 100 cc. of sterile water. Thus the final dilution was 1:100,000.

Dilution plates were made by adding a single cubic centimeter of the suspension from the last of the dilution series to a test tube containing approximately 15 cc. of Conn's sodium-asparaginate agar (71). The temperature of the agar when the suspension was added was between

40 and 45°C., that is, just above the solidifying point. The agar-suspension mixture was rotated vigorously and poured into sterile petri dishes. From five to seven dilutions were poured from each soil.

These plates were incubated at 28°C. for eight to ten days, after which the Actinomyces colonies developing on each plate were checked by microscopic observation and the number recorded. The individual colonies were isolated to oatmeal agar slants and incubated at room temperatures. In order to make the sampling randomized, all the cultures were picked up from a given plate or from certain portions of it.

Laboratory Studies:

The test for antibiosis against a Pythium arrhenomanes isolate known to be pathogenic to sugarcane was made by streaking four isolates of the Actinomyces about the edge of a 9 cm. petri dish on Czapek's modified agar (71). These were incubated at 28°C. for 48 hours before a plug of agar 7 mm. in diameter from an actively growing Pythium culture was placed in the center of the plate, equidistant from all four Actinomyces streaks. This procedure allows for approximately 2.5 cm. between the Pythium inoculum and the Actinomyces streaks.
After 7 days of incubation at 28°C., the distance between the Actinomyces and the foremost edge of the Pythium culture was determined and recorded in millimeters. At that time colonies that failed to show any antibiosis were overgrown by the Pythium mycelium. Actinomyces isolates showing any inhibition of the mycelium were retested in a similar manner, and all those that failed to show any inhibition were discarded. Any of the isolates with an inhibitory distance of 10 mm. in either of these two preliminary tests were retested by streaking a single isolate to a petri dish. The Pythium inoculum was placed on

the opposite side of the petri dish 48 hours later. This allowed for approximately 5.0 cm. between the Actinomyces and the Pythium inocula. Incubation time and temperature were the same as in the initial tests. This single culture per plate prevented any interaction between the isolates, and also made possible the determination of the relative inhibitory distances of the more active cultures.

In order to describe the antibiotic nature of any Actinomyces population, two arbitrary terms were defined and used. The antibiotic index was defined as the calculated mean inhibition of the Actinomyces isolates tested from any population sample. The individual Actinomyces isolates tested were separated into one of five classes, depending upon their average inhibitory distance. These classes were 0, 1-5, 6-10, 11-15, and 16 or more millimeters inhibition. To facilitate calculation, 0, 3, 8, 13, and 18 were arbitrarily taken to represent the mean value of each class. Then the sum of the products of frequency and class value divided by the total number of isolates tested gives the antibiotic index of the population sample.

Some of the Actinomyces isolates failed to grow when transferred to the oatmeal agar slant or were contaminated. Also, isolates were taken from different numbers of plates in some instances. Thus the number of isolates tested was not always proportional to the Actinomyces populations. To account for this difference, an antibiotic value was calculated for each soil sample by multiplying the antibiotic index by the number of thousands of Actinomyces per gram soil. The antibiotic value as thus derived was considered as a measure of the antibiotic activity of the Actinomyces in a given soil.

The pH determination of each soil sample was made as follows: 50 grams of air dried soil sieved through a 4-mesh screen were weighed into

well rinsed beakers and 100 cc. of water added. Duplicate samples were weighed out for each soil sample. These were allowed to set for a period of 36 to 48 hours. During this time they were stirred four to five times, but otherwise kept covered. The determinations were made with a Beckman potentiometer just following a vigorous stirring of the sample. The average of two readings was calculated and recorded.

To determine the difference in Actinomyces associated with the cane roots and those in the soil, a larger sample of soil was dug up and the cane roots removed from it along with the normal amount of soil as sample. The cane roots were left in the soil sample until just before dilutions were made. The roots were then separated from the soil by a 4-mesh soil screen. All the dry soil that could be shaken from the roots was removed. The soil was then diluted according to standard procedure. The roots were weighed, placed in a large mortar, moistened with water, and ground with a pestle until the cortex of the roots was well macerated. Then the total contents of the mortar was rinsed into a flask and enough water added to bring the dilution, based upon the weight of the roots, up to 1:100. Further dilutions were made according to standard procedure for the soil dilutions. Thus the final dilution was 1:1,000,000. A dilution of 1:10,000,000 was also made of the last four samples of roots. Isolation and testing procedures were the same as those used for the soil Actinomyces.

Greenhouse Studies:

The soil used in all greenhouse studies, unless otherwise stated, was a medium light soil from the Sugarcane Experiment Station, Baton Rouge, Louisiana. All culture work was done in 6-inch porous clay pots. The potted soil was kept under light steam pressure for $2\frac{1}{2}$ to 4 hours to sterilize.

All Actinomyces cultures were grown on nutrient broth with 0.5 per cent dextrose. The Pythium culture was grown on plates of Czapek's agar. Sterile media were always added to the checks.

The amendments added to the soil consisted of commercial wheat flour, C. P. sodium nitrate, dried shredded cane trash, and a commercial table syrup. These amendments were well mixed into the soil just before the Actinomyces or Pythium were added. The organisms were thoroughly mixed into the top 2½ to 3 inch layer of soil in the pots. Individual new pot labels were used for mixing in isolate in order to prevent cross-contamination.

The cultures of soil were incubated under greenhouse conditions for 7 to 10 days before planting. The White Tuxpan variety of corn was used in these studies. Ten grains were planted to each pot, and, unless otherwise stated, all plants developing from these were allowed to grow. All greenhouse experimental pot cultures were watered with tap water, although recent studies indicate that this results in an accumulation of alkaline salts.

The height of the individual corn plants was determined and recorded in inches. This was considered to indicate the severity of the root rot; however, later studies indicated that this criterion was not as satisfactory as observation and classification of the diseased condition of the washed roots.

Actinomyces added to Field Soils:

The Actinomyces cultures were grown on nutrient broth with 0.5 per cent dextrose. Before application, the Actinomyces were suspended by putting them in a Waring blender for a few seconds. Then these were further diluted with tap water and sprinkled on the seedpieces after

they were placed in the furrow or in the middles between the soybean crop just before they were turned under. In the test in which three isolates were applied together, the Actinomyces were grown separately, and they were then filtered from the broth on the same filter paper. The filter paper and Actinomyces were then well mixed with the flour or with sand as a check. This mixture was scattered on the seed pieces and adjacent soil just before the cane was covered.

RESULTS OF THE SURVEY

The Actinomyces counts as derived from individual soil samples varied considerably, as would be expected when such a wide range of soil types was included and the sampling extended through the different seasons. The data for each determination are shown in Tables I and II. These tables include, in addition to the survey data, the results of isolation from soils receiving various treatments and inoculations in field and greenhouse studies.

Table I includes the counts of the Actinomyces colonies developing on each of the dilution plates, the number of thousands of Actinomyces per gram of soil based upon these dilution counts and the number of isolates tested that occur in each of the inhibition classes. The antibiotic index is also shown here. A detailed description of each sample is given in Table II, including the date collected, the date plated out, the population per gram of soil, and the antibiotic index and value.

The average number of Actinomyces colonies that developed on the dilution plates of each sample varied considerably. Expressed in terms of the Actinomyces population per gram of soil, they varied from 135,000 to 4,375,000. There were also differences between the determinations for samples taken at different times. This variation makes comparison between the population counts of any two samples rather difficult. However, when the comparisons are based on the average of several determinations for each group being compared, they are considered

Table 1. The Actinomyces Counted and Tested from Various Soil Samples and their Distribution in the Different Inhibition Classes.

Soil No.	Plantation	Soil type*	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
1	Glenwood	M	10	11	9	2	6	6	1	10	700**	37	14	2	2	0	55	1.53
2	Glenwood	L	10	3	2	1	0	2	7	2	225**	13	4	0	0	0	17	0.71
3	J. Lanier	M	10	5	6	10	1	3	19	8	1,012**	52	17	7	4	2	82	2.38
4	J. Lanier	L	10	6	10	6	6	10	8	9	775**	29	21	9	2	0	61	2.64
5	J. Lanier	H	10	3	2	3	1	2	2	4	212**	12	2	3	0	0	17	1.82
6	L. Texas	L	10	6	11	7	8	7	9	9	800**	40	9	7	2	2	60	2.42
7	L. Texas	M	10	0	0	2	2	0	3	2	125**	3	3	0	0	0	6	1.50
8	L. Texas	H	10	14	13	8	11	11	5	12	1,100**	61	19	5	2	1	88	1.60
25	DeGreaux	L	10	9	14	10	4	-	-	-	925	26	6	7	3	0	42	2.69
26	McCall	L	10	10	11	11	12	-	-	-	1,100	33	5	4	2	1	45	2.02
27	Cinclare	L	10	4	11	7	10	-	-	-	800	19	9	3	0	0	31	1.64
28	Cinclare	H	10	2	2	5	3	-	-	-	300	11	1	0	0	0	12	0.25
29	Cinclare	H	10	8	11	6	7	-	-	-	800	27	1	1	0	0	29	0.38
30	Albania	L	10	7	7	11	10	-	-	-	875	15	6	3	1	0	25	0.68
31	Humus	-	0.1	3	3	3	2	-	-	-	27,500							
32	J. Lanier	L	10	1	2	2	5	-	-	-	250	3	1	1	1	0	6	4.00
33	J. Lanier	H	10	1	0	2	4	-	-	-	175	5	2	0	0	0	7	0.86
34	Caffery	L	10	6	8	12	10	-	-	-	900	19	0	5	1	2	27	3.30
35	Caffery	H	10	2	3	6	2	-	-	-	325	11	2	0	0	0	13	0.46
36	L. Texas	L	10	6	4	4	12	-	-	-	650	20	3	1	0	1	25	1.40
37	L. Texas	H	10	7	2	7	9	-	-	-	625	14	1	2	4	1	22	4.04
38	Glenwood	L	10	7	11	6	7	-	-	-	775	18	4	3	1	1	27	2.48
39	Glenwood	H	10	4	0	2	2	-	-	-	200	4	0	1	0	0	5	1.60
40	Albania	L	10	3	4	8	12	-	-	-	675	20	1	3	3	1	28	3.11
41	Albania	L[1]	10	25	12	19	14	-	-	-	1,750	46	5	4	2	1	58	1.57
42	Maryland	L	10	5	8	6	9	-	-	-	700	18	2	0	0	1	21	1.14

* Legend. L=light, H=heavy, M=mixed, Ya=Yahola, Lin.=Lintonia, Ol=Olivier, F.P.M.= Filter press added to the soil, Gh=light soil in greenhouse studies.

** Based upon counts of eight plates.

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
43	Maryland	L2	10	30	33	16	17	-	-	-	2,400	51	5	3	0	0	59	0.66
44	Caffery	L	10	27	24	24	23	-	-	-	2,450	62	6	2	0	1	71	0.73
45	Caffery	H	10	10	9	8	11	-	-	-	950	25	0	1	0	1	27	0.96
46	Glenwood	L	10	8	18	10	15	-	-	-	1,275	31	3	3	0	2	39	1.77
47	Glenwood	H	10	2	8	7	9	-	-	-	650	18	1	0	0	1	20	1.05
48	Greenwood	L	10	4	11	7	7	-	-	-	725	20	4	1	0	1	26	2.04
49	Greenwood	H	10	16	24	16	18	-	-	-	1,650	45	1	2	1	1	50	1.25
50	Albania	L	10	10	10	5	10	-	-	-	875	18	1	1	1	0	21	1.14
51	Albania	L1	10	18	15	14	11	-	-	-	1,450	24	4	0	0	0	28	0.43
52	J. Lanier	L	10	2	7	7	3	7	8	-	567	22	4	3	1	2	32	3.66
53	J. Lanier	H	10	3	5	4	4	6	3	-	425	19	0	1	0	0	20	0.40
54	L. Texas	L	10	14	7	12	14	36	10	-	1,550	52	10	1	4	0	67	1.34
55	L. Texas	H	10	1	1	6	10	13	4	-	583	24	1	3	1	0	29	1.38
56	Glenwood	FPM	10	4	1	1	2	3	2	-	100	9	2	0	0	0	11	1.59
57.	Cora-Texas	FPM	10	0	1	1	1	2	1	-	217	4	1	1	0	0	6	0.54
58	Morgan C.	L	10	4	9	8	7	5	6	-	650	32	1	1	0	0	34	0.38
59	Billeaud	Lin.	10	9	11	5	11	10	6	-	866	35	6	0	1	0	42	0.74
60	Maryland	L	10	10	5	1	9	5	10	-	666	29	2	2	2	0	35	1.37
61	Maryland	L2	10	6	13	8	13	-	-	-	1,000	36	1	1	0	1	39	0.74
62	Greenwood	L	10	10	15	15	9	-	-	-	1,225	27	5	10	3	0	45	2.98
63	Greenwood	H	10	7	11	9	8	-	-	-	875	15	1	0	0	1	17	1.23
64	L. Texas	L	10	15	10	10	11	-	-	-	1,150	34	1	5	0	0	40	1.08
65	L. Texas	H	10	10	12	10	11	-	-	-	1,075	26	11	2	1	0	40	1.55
66	Caffery	L	10	20	17	21	10	-	-	-	1,700	49	7	0	2	0	58	0.81
67	Caffery	H	10	13	18	20	12	17	19	-	1,650	79	3	2	4	2	90	1.25
68	Albania	L	10	19	17	15	17	-	-	-	1,700	42	1	3	2	2	50	1.78
69	Albania	L1	10	13	23	27	21	-	-	-	2,100	59	3	2	1	0	65	0.58
70	Montegut	L	10	16	14	17	8	-	-	-	1,375	25	2	1	1	1	30	1.50
71	Montegut	H	10	24	16	26	22	-	-	-	2,200	79	4	2	0	1	86	0.54
72	Youngsville	Cl	10	16	14	22	15	-	-	-	1,675	48	2	5	1	0	56	1.05
73	Billeaud	Lin.	10	14	14	9	7	-	-	-	1,100	25	2	2	2	3	34	2.56

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
74	Morgan C.	L	10	17	19	16	17	-	-	-	1,725	42	3	4	1	1	51	1.41
75	Humus	-	1	36	39	45	34	44	-	-	39,600	-	-	-	-	-	-	-
76	Shirley	Ya	10	12	15	16	16	-	-	-	1,475	30	10	11	3	2	56	3.45
77	Meeker	Ya	10	13	10	8	11	-	-	-	1,050	15	10	10	1	1	37	3.81
78	J. Lanier	L	10	14	21	22	17	-	-	-	1,850	46	2	8	0	1	57	1.54
79	J. Lanier	H	10	6	6	11	6	-	-	-	725	16	3	3	4	0	26	3.40
80	Cinclare	L	10	20	22	15	14	-	-	-	1,775	38	4	6	1	0	49	1.49
81	Cinclare	H	10	16	14	7	13	-	-	-	1,250	41	0	2	1	0	44	0.66
82	Glenwood	L	10	19	14	13	16	-	-	-	1,550	47	3	6	2	2	60	1.98
83	Glenwood	H	10	10	9	9	3	-	-	-	775	22	1	0	3	1	27	2.22
84	Glenwood	L	10	9	21	12	23	-	-	-	1,625	46	8	3	2	0	59	1.25
85	Reserve	L	10	11	13	16	10	-	-	-	1,250	30	5	6	4	1	46	2.89
86	Reserve	H	10	14	10	13	12	-	-	-	1,225	40	0	1	6	1	48	2.17
87	Reserve	H	10	4	10	8	12	-	-	-	850	31	2	0	1	0	34	0.56
88	Cutoff	Muck	1	6	6	6	9	2	-	-	4,337	49	6	0	3	0	58	0.98
	"	"	10	34	38	-	-	-	-	-								
89	Calhoun	-	10	25	23	27	25	-	-	-	2,500	40	8	9	3	4	64	3.24
90	LSU - Check	L	10	14	25	22	-	-	-	-	2,033	26	2	3	0	1	32	1.34
91	LSU -Gr-2-8	L	10	26	16	14	35	-	-	-	2,275	50	7	1	0	0	58	0.50
92	LSU - Check	L	10	23	16	16	24	-	-	-	2,050	16	0	1	0	0	17	0.47
93	LSU - Check	L	10	17	19	26	29	-	-	-	2,067	23	4	1	0	0	28	0.71
94	LSU - Gr-2-8	L	10	30	34	34	-	-	-	-	3,266	21	7	2	1	0	31	1.61
95	LSU - Check	L	10	12	13	13	23	-	-	-	1,525	13	1	1	0	0	15	0.73
96	LSU - Check	L	10	23	14	25	20	-	-	-	2,050	28	0	1	2	0	31	1.10
97	LSU - Check	L	10	20	24	25	38	-	-	-	2,675	25	0	0	0	0	25	0.00
98	LSU - Check	L	10	24	22	17	23	-	-	-	2,150	20	1	0	1	0	32	0.73
99	LSU - Check	L	10	23	18	21	29	-	-	-	2,275	35	3	2	0	0	40	0.68
100	Youngsville	Cl	10	7	11	17	10	-	-	-	1,125	18	0	3	5	2	28	4.46
101	Billeaud	Lin.	10	3	4	4	4	-	-	-	375	11	1	0	0	0	12	0.35
102	Morgan C.	L	10	7	9	10	7	-	-	-	825	18	4	3	1	0	26	1.64
103	Albania	L	10	9	11	13	14	-	-	-	1,175	23	6	9	1	0	39	2.64

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
104	Caffery	L	10	25	30	21	26	-	-	-	2,300	56	7	5	2	2	72	1.71
105	Caffery	H	10	12	12	15	8	-	-	-	1,175	36	2	0	0	0	38	0.16
106	Maryland	L	10	19	14	12	17	-	-	-	1,650	39	3	5	4	0	51	1.98
107	Maryland	H[2]	10	17	6	12	18	-	-	-	1,325	25	4	4	0	0	33	1.32
108	Glenwood	L	10	21	21	12	21	-	-	-	1,875	37	4	10	4	2	57	3.16
109	Glenwood	H	10	8	12	17	15	-	-	-	1,550	45	1	2	3	0	51	1.14
110	Montegut	L	10	27	23	19	31	-	-	-	2,500	59	6	6	5	2	78	2.17
111	Montegut	H	10	27	25	20	23	-	-	-	2,375	64	4	2	1	0	71	0.58
112	Montegut	H	10	17	20	15	19	-	-	-	1,775	44	3	7	4	7	65	3.74
113	L. Texas	H	10	14	17	13	13	-	-	-	1,425	32	2	6	1	10	51	4.84
114	L. Texas	L	10	13	8	6	12	-	-	-	1,225	26	6	2	2	4	40	3.30
115	L. Texas	H	10	19	16	13	13	-	-	-	1,525	35	3	8	3	1	50	2.60
116	Cinclare	L	10	15	23	15	19	-	-	-	1,800	41	8	8	1	1	59	2.02
117	Cinclare	H	10	34	30	22	28	-	-	-	2,850	60	1	6	1	1	69	1.19
118	Greenwood	L	10	10	4	6	6	-	-	-	650	8	4	8	3	0	23	5.00
119	Greenwood	H	10	8	12	5	8	-	-	-	825	19	1	1	1	3	25	3.12
120	J. Lanier	L	10	12	11	14	11	-	-	-	1,350	32	3	0	1	1	37	1.08
121	J. Lanier	H	10	18	18	21	18	-	-	-	1,875	48	4	3	0	2	57	1.26
122	Reserve	L	10	10	12	13	10	-	-	-	1,125	20	0	6	3	1	30	3.50
123	Reserve	H	10	31	15	18	20	-	-	-	2,100	41	6	4	1	3	55	2.13
124	Shirley	Ya	10	18	21	18	23	-	-	-	2,000	44	8	9	5	0	66	2.44
125	Meeker	Ya	10	12	10	12	14	-	-	-	1,200	19	5	7	4	5	40	5.38
126	Melrose	Ya	10	8	13	11	14	-	-	-	1,150	13	1	1	2	0	17	2.18
127	Cinclare	L	10	14	11	24	15	-	-	-	1,600	46	2	5	7	0	60	2.28
128	Cinclare	H	10	18	17	11	22	-	-	-	1,700	56	1	6	2	2	67	1.69
129	Cora Texas	TPM	10	26	24	23	23	-	-	-	2,400	71	6	1	2	1	81	0.86
130	Glenwood	L	10	27	22	20	23	-	-	-	2,300	54	11	7	4	4	80	2.66
131	Glenwood	H	10	26	23	17	21	-	-	-	2,175	56	11	3	1	1	72	1.31
132	J. Lanier	L	10	7	6	7	8	8	6	-	700	24	4	2	3	0	33	2.03
133	J. Lanier	H	10	20	25	17	19	-	-	-	2,025	45	9	4	2	0	60	1.41
134	L. Texas	L	10	18	16	16	18	-	-	-	1,700	36	9	5	10	2	62	3.75

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
135	L. Texas	H	10	16	17	12	16	-	-	-	1,525	42	5	6	2	0	55	1.62
136	Greenwood	L	10	23	31	21	23	-	-	-	2,450	48	5	5	5	2	65	2.40
137	Greenwood	H	10	29	22	25	25	-	-	-	2,525	63	5	7	2	4	81	2.09
138	Morgan C.	L	10	29	19	18	31	-	-	-	2,425	65	11	2	1	0	78	0.78
139	Caffery	L	10	40	30	39	34	-	-	-	3,575	101	16	2	3	2	124	1.12
140	Caffery	H	10	10	10	14	13	-	-	-	1,175	38	3	3	1	0	45	1.02
141	Albania	L	10	16	16	14	16	-	-	-	1,550	43	7	3	1	0	55	1.06
142	Billeaud	Lin.	10	11	12	19	7	-	-	-	1,225	33	10	2	0	0	45	1.02
143	Youngsville	FPM	10	5	8	6	6	-	-	-	625	15	2	0	0	0	17	0.35
144	Youngsville	Ol[3]	10	14	11	18	12	-	-	-	1,375	35	9	2	0	0	47	0.91
145	Youngsville	Ol[4]	10	3	2	3	3	0	1	-	200	8	2	2	0	0	12	1.83
146	Youngsville	Ol	10	20	19	16	25	-	-	-	2,000	51	14	3	2	0	70	1.31
147	Shirley	Ya	10	10	4	7	8	6	9	-	733	25	6	5	2	1	39	2.62
148	Meeker	Ya	10	7	7	8	6	7	7	-	700	20	11	3	3	0	37	2.59
149	Reserve	L	10	24	25	21	27	-	-	-	2,425	56	9	6	4	1	76	1.86
150	Reserve	H	10	9	12	5	6	-	-	-	800	12	1	0	1	1	15	2.27
151	LSU - Gr-2-8	L	10	24	25	22	28	-	-	-	2,475	37	3	4	0	1	45	1.31
152	LSU - Check	L	10	32	21	17	21	-	-	-	2,375	44	8	8	0	0	60	1.47
153	LSU - Gr-2-8	L	10	29	29	33	26	-	-	-	2,925	46	10	10	3	2	71	2.65
154	LSU - Check	L	10	34	26	33	28	-	-	-	3,025	63	6	13	7	2	91	2.74
155	LSU - 52-20	H	10	20	19	23	17	26	24	-	2,117	66	15	4	1	0	86	1.05
156	LSU - Check	H	10	24	21	27	26	-	-	-	2,450	72	9	3	1	1	86	0.95
157	LSU-Act.+F	H	10	13	15	15	13	8	9	10	1,186	48	7	1	0	0	48	0.60
158	LSU-Act.+S	H	10	22	18	13	24	21	20	-	1,917	62	18	3	5	3	91	2.16
159	LSU - Ster.[5]	Gh	10	0	0	0	0	0	0	2	13[6]	2	0	0	0	0	2	0.00
160	LSU-Unster.	Gh	10	20	14	23	21	20	22	-	2,667	56	17	17	1	1	92	2.37
161	LSU - SB1	Gh	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
162	LSU - SB2	Gh	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
163	LSU - SB3	Gh	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
164	LSU - SB4	Gh	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
165	LSU - B1	Gh	10	24	17	25	22	-	-	-	2,200	39	4	7	8	4	62	3.94

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index.
166	LSU - B2	Gh	10	34	35	35	28	-	-	-	3,300	39	10	13	9	9	79	5.23
167	LSU - B3	Gh	10	20	18	13	19	20	-	-	1,800	46	13	13	6	0	78	2.83
168	LSU - B4	Gh	10	20	15	12	15	21	-	-	1,660	43	8	9	8	2	70	3.37
169	LSU -A(ck.)	Gh	10	13	20	17	19	14	-	-	1,660	49	8	5	1	0	63	1.22
170	LSU - SAB	Gh	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
171	LSU - Gr-2-87	Gh	10	3	2	1	3	0	5	-	333	3	1	2	2	1	9	7.00
172	LSU - 52-20	Gh	10	2	6	5	4	11	2	4	486	4	13	4	3	5	29	6.90
173	LSU - 100-32	Gh	10	1	0	0	1	3	1	2	114	0	0	0	0	1	1	18.00
174	LSU - 108-8	Gh	10	42	32	41	-	-	-	-	3,833	1	2	0	0	35	38	16.74
175	LSU - 87-5	Gh	10	6	4	7	8	4	8	2	586	39	0	0	0	0	39	0.00
176	LSU - 40-5	Gh	10	5	4	4	6	8	1	7	500	8	8	6	5	1	28	5.54
177	LSU - 110-67	Gh	10	2	2	3	7	11	5	7	529	3	2	4	0	1	10	5.60
178	LSU - 113-12	Gh	10	110	119	-	-	-	-	-	11,450	11	11	16	3	2	43	5.49
179	LSU - 82-37	Gh	10	30	44	29	-	-	-	-	3,433	15	11	12	4	8	50	6.50
180	LSU - 42-2	Gh	10	0	3	2	0	3	0	2	143	4	1	3	0	0	8	3.38
181	LSU 124-1	Gh	10	279	316	-	-	-	-	-	29,750	15	19	12	5	0	51	4.27
182	LSU - 119-11	Gh	10	6	4	1	2	2	5	4	343	4	2	6	1	0	13	5.15
183	LSU - M-25	Gh	10	4	2	2	2	2	3	2	0	0	1	9	3	1	14	9.43
184	LSU - Check	Gh	10	2	0	4	5	0	2	3	229	6	0	0	0	0	6	0.00
185	Milleaud	Lin.	10	3	5	8	11	-	-	-	675	19	4	0	1	0	24	1.04
186	Youngsville	Cl	10	23	14	26	25	-	-	-	2,900	60	12	1	3	0	76	1.09
187	Glenwood	L	10	12	20	16	17	-	-	-	1,625	45	9	3	2	1	60	1.58
188	Glenwood	H	10	8	11	14	3	-	-	-	900	22	3	4	2	0	31	2.16
189	Shirley	Ya	10	28	19	21	9	-	-	-	1,925	42	6	3	2	1	54	1.59
190	Meeker	Ya	10	17	14	13	12	-	-	-	1,400	30	11	5	1	0	47	1.83
191	Meeker	Ya	10	27	19	20	25	-	-	-	2,275	64	20	2	1	0	87	1.02
192	Crowley	Rice	10	28	29	14	25	-	-	-	2,400	60	8	4	3	0	75	1.27
193	Greenwood	L	10	39	35	41	23	-	-	-	3,450	40	18	3	3	0	64	1.83
194	Greenwood	H	10	12	13	15	16	-	-	-	1,400	41	4	1	4	0	50	1.44
195	L. Texas	L	10	30	33	24	33	-	-	-	2,500	53	23	2	9	2	89	2.67
196	L. Texas	L	10	30	38	24	29	-	-	-	2,775	68	11	4	2	0	85	1.07

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
197	Cinclare	L	10	28	27	33	35	-	-	-	3,075	58	8	2	1	1	70	1.01
198	Cinclare	H	10	31	26	33	31	-	-	-	3,025	59	5	4	2	0	70	1.04
199	Caffery	L	10	31	40	34	36	-	-	-	3,525	62	6	1	1	0	70	0.56
200	Caffery	H	10	15	25	20	15	-	-	-	1,875	41	3	4	4	0	52	1.85
201	Albania	L	10	48	43	40	43	-	-	-	4,375	50	5	7	5	1	68	2.26
202	Albania	H	10	31	18	21	18	-	-	-	2,200	51	3	4	3	0	61	1.31
203	Reserve	L	10	28	32	24	24	-	-	-	2,950	39	6	3	2	0	50	1.36
204	Reserve	H	10	21	18	15	20	-	-	-	1,850	35	12	4	0	1	52	1.65
205	LSU - 1[8]	Lin.	10	63	62	57	-	-	-	-	6,200	15	6	7	2	0	30	3.33
206	LSU - 2	Lin.	10	131	135	114	-	-	-	-	12,670	2	1	1	1	0	5	4.80
207	LSU - 3	Lin.	10	91	72	82	-	-	-	-	8,670	24	8	5	5	2	44	3.75
208	LSU - 4	Lin.	10	70	73	77	-	-	-	-	7,333	44	9	8	1	1	63	1.94
215	Cinclare	L	10	3	1	8	4	0	-	-	320	9	0	1	0	0	10	0.80
216	Cinclare	H	10	9	8	11	7	5	-	-	1,000	15	2	3	1	0	21	2.05
217	Cora Texas	FPM	10	17	29	23	19	-	-	-	2,200	20	1	1	1	1	24	1.75
218	Glenwood	L	10	5	4	11	6	-	-	-	650	6	1	1	1	5	14	8.14
219	Glenwood	H	10	13	15	6	17	19	-	-	1,400	26	11	5	1	0	43	2.00
220	J. Lanier	L	10	5	2	7	7	9	7	6	614	29	0	2	4	0	35	1.94
221	J. Lanier	H	10	5	6	11	10	8	12	-	867	22	2	3	0	1	28	1.71
222	L. Texas	L	10	13	16	20	12	-	-	-	1,525	31	3	2	0	0	36	0.69
223	L. Texas	H	10	10	7	10	7	13	10	-	950	27	0	0	1	0	28	0.46
224	L. Texas[9]	L	10	17	9	14	11	20	-	-	1,420	39	6	2	0	0	47	0.72
225	Greenwood	L	10	20	28	21	28	-	-	-	2,425	57	3	6	2	1	69	1.46
226	Greenwood	H	10	2	12	3	4	5	2	-	467	10	2	2	0	0	14	1.57
233	Morgan C.	L	10	7	13	11	10	11	12	8	1,029	36	2	2	2	0	42	1.14
234	Caffery	L	10	6	5	7	9	19	10	10	943	29	6	1	3	0	39	1.67
235	Caffery	H	10	6	2	2	4	2	0	3	357	9	1	0	0	0	10	0.30
236	Albania	L	10	10	7	4	6	11	14	-	867	35	7	3	2	1	48	1.85
237	Albania	H	10	16	11	10	10	13	8	-	1,133	34	4	4	1	0	33	1.73
238	Billeaud	Lin.	10	4	4	2	7	6	3	11	529	21	6	0	1	0	28	1.11
239	Youngsville	CL	10	6	6	8	9	6	7	6	686	19	4	2	0	1	26	1.77

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
240	Youngsville	Ol	10	2	2	2	9	8	7	3	429	17	5	1	0	0	23	1.00
241	Reserve	L	10	6	9	15	5	15	7	-	950	27	5	4	2	0	38	1.92
242	Reserve	H	10	14	14	20	22	19	21	-	1,833	48	3	0	0	1	52	0.52
243	Meeker	Ya	10	2	2	8	7	6	3	5	471	13	4	3	0	0	20	1.80
244	Meeker	Ya	10	5	13	14	6	7	7	10	686	28	10	3	1	1	43	1.98
245	Bubenzer	Ya	10	11	15	9	11	5	15	9	1,100	40	6	5	2	1	54	1.89
245A	Cane roots	245	1	41	32	34	38	39	-	-	36,800	47	8	10	3	0	68	2.10
246	Shirley	Ya	10	14	5	4	4	7	5	4	614	7	2	0	1	0	10	1.90
247	LSU - Check	L		5	7	9	6	1	6	9	614	22	3	2	0	0	27	0.93
248	LSU - Gr-2-8	L		10	5	6	6	7	7	5	557	13	3	0	1	0	17	1.29
249	241 - P. Agar-			17	14	17	12	-	-	-	1,500	30	6	5	1	0	42	1.69
250	243 - P. Agar-			5	7	5	6	10	-	-	550	11	0	3	1	2	17	4.29
251	246 - P. Agar-			11	14	11	8	-	-	-	1,100	17	6	4	0	0	27	1.85
295	L. Texas	L	10	17	14	15	13	19	12	-	1,500	43	9	8	0	1	61	1.79
296	Cane roots	295	1	4	6	4	7	3	0	-	4,000	23	1	1	0	2	27	1.74
	" "	"	0.1	2	0	0	0	1	0	0	4,286	-	-	-	-	-	-	-
297	Caffery	L	10	17	18	29	14	30	18	32	2,257	45	4	1	5	0	55	1.54
298	Cane roots	297	1	6	12	3	5	7	6	6	6,429	26	3	2	0	0	31	0.81
	" "	"	0.1	3	1	3	3	0	0	-	16,667	-	-	-	-	-	-	-
299	Billeaud	Lin.	10	32	37	34	30	37	4	-	2,733	41	10	3	2	2	58	2.00
300	Billeaud	Lin.	10	4	9	5	9	12	15	7	871	47	3	3	0	0	53	0.62
301	Cane roots	300	1	11	11	6	5	18	10	9	10,000	40	11	1	0	0	52	0.79
	" "	"	0.1	1	1	2	1	0	2	0	10,000	-	-	-	-	-	-	-
302	Billeaud	Lin.	10	8	10	8	6	9	10	-	850	31	3	1	1	2	38	1.74
303	Billeaud	Lin.	10	10	23	17	16	13	20	8	1,529	49	14	1	0	0	64	0.78
304	Youngsville	Ol	10	23	10	17	10	4	14	16	1,200	57	8	0	2	0	67	0.75
305	Youngsville	Ol	10	16	34	21	29	23	23	32	2,686	37	10	3	7	1	58	2.81
306	Bubenzer	Ya	10	10	10	7	5	8	7	10	814	37	3	6	1	0	47	1.49
307	Cane roots	306	1	7	11	11	14	9	13	-	10,822	38	13	3	1	0	55	1.37
	" "	"	0.1	3	3	5	4	3	2	5	35,714	-	-	-	-	-	-	-
308	Shirley	Ya	10	13	15	15	14	16	15	16	1,657	57	3	1	4	2	67	1.57

Table 1 (continued)

Soil No.	Plantation	Soil type	Dil. ppm.	Actinomyces per plate 1	2	3	4	5	6	7	1000/ gram	Millimeters inhibition 0	1-5	6-10	11-15	16-	Tot.	Anti. Index
309	Shirley	Ya	10	8	15	17	9	8	10	13	1,143	54	6	3	5	0	68	1.57
310	Meeker	Ya	10	6	7	7	6	2	6	-	567	21	9	2	2	0	34	2.03
311	Meeker	Ya	10	24	23	28	22	27	-	-	2,480	36	14	11	0	2	63	2.63
312	Meeker	Ya	10	9	10	8	19	9	12	13	1,072	54	5	2	2	0	63	0.90

1. Light soil but poorly drained.
2. Light, but dark colored soil with very much organic matter.
3. Soil taken from around stubbles; that is, from the top 4 to 5 inches of soil.
4. Soil taken from below the stubble; that is, from the plow sole after stubble was turned out.
5. Soil from greenhouse studies receiving various treatments.
6. Population determined by averaging 15 plates.
7. Isolate numbers of Actinomyces added to sterilized soil - greenhouse studies.
8. The effect of different sources of nitrogen upon Actinomyces in Lintonia light soils from LSU Horticultural Experiment Station. 1 = check, 2 = rice straw, 3 = dried blood, and 4 = NH_4NO_3. All were added at the rate of 140 pounds nitrogen per acre. Inoculated for 53 days at optimum conditions in laboratory.
9. Sample taken from severe root rot area, almost complete crop failure.

Table II. Description of Soil Samples and Antibiotic Activity of Actinomyces Population.

Soil No.	Plantation, with notes for each sample	Soil type[1]	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
1	Glenwood	M	6.8	3/1/46	3/6/46	700	1.53	1,071
2	Glenwood	L	7.8	"	"	225	0.71	160
3	J. Lanier	M	6.6	"	"	1,012	2.38	2,408
4	J. Lanier	L	7.4	"	"	775	2.64	2,046
5	J. Lanier, black stiff land	H	6.5	"	"	212	1.82	386
6	L. Texas, C.P. 36/105	L	6.7	"	"	800	2.42	1,936
7	L. Texas, black land, C.P. 29/120 plant	M	6.7	"	"	125	1.50	188
8	L. Texas, Sharkey clay, C.P. 34/120 plant	H	6.8	"	"	1,100	1.60	1,760
25	DeGreaux	L	6.6	3/21/46	3/26/46	925	2.69	2,488
26	McCall	L	6.4	"	"	1,100	2.02	2,222
27	Cinclare	L	6.6	4/16/46	4/19/46	800	1.64	1,312
28	Cinclare	H	6.8	"	"	300	0.25	75
29	Cinclare	H	6.7	"	"	800	0.38	304
30	Albania	L	8.0	"	"	875	0.68	595
31	Humus, prepared humus from Dr. Lutman	-	-	-	"	2,750	1.21	3,328
32	J. Lanier	L	6.8	3/21/46	4/22/46	250	4.00	1,000
33	J. Lanier	H	6.4	"	"	175	0.86	150
34	Caffery	L	6.8	"	"	900	3.30	2,970
35	Caffery	H	6.4	"	"	325	0.46	150
36	L. Texas	L	6.7	"	"	650	1.40	910
37	L. Texas	H	6.7	"	"	625	4.04	2,525
38	Glenwood	L	7.2	"	"	775	2.48	1,922
39	Glenwood	H	7.0	"	"	200	1.60	320
40	Albania	L	5.5	"	"	675	3.11	2,099
41	Albania, poorly drained	L	6.1	"	"	1,750	1.57	2,748
42	Maryland, light colored, silt loam	L	5.9	5/10/46	5/12/46	700	1.14	798
43	Maryland, dark loam	L	6.7	"	"	2,400	0.66	1,584
44	Caffery	L	6.5	"	"	2,450	0.73	1,788
45	Caffery	H	6.5	"	"	950	0.96	912
46	Glenwood	L	6.6	"	"	1,275	1.73	2,206
47	Glenwood	H	7.0	"	"	650	1.05	682

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
48	Greenwood	L	7.2	5/10/46	5/12/46	725	2.04	1,479
49	Greenwood	H	6.7	"	"	1,850	1.25	2,312
50	Albania	L	5.7	"	"	875	1.14	998
51	Albania, poorly drained	L	5.7	"	"	1,450	0.43	624
52	J. Lanier, plant cane	L	7.2	"	"	567	2.66	1,508
53	J. Lanier, C.P. 34/120, plant cane	H	6.7	"	"	425	0.40	170
54	L. Texas	L	6.8	"	"	1,550	1.34	2,077
55	L. Texas, C.P. 34/120, low area	H	7.4	"	"	583	1.38	804
56	Glenwood, FPM 1-2" on soil, fall 1945	FPM	6.4	"	"	100	1.59	159
57	Cora Texas, FPM 4-6" - no soil in sample	FPM	6.3	"	"	217	0.54	117
58	Morgan City, Iberia loam	L	7.5	"	"	650	0.32	208
59	Billeaud, sandy loam, Co. 290 stubble	Lin.	5.6	"	"	866	0.74	641
60	Maryland, light colored silt loam	L	5.1	5/30/46	6/7/46	666	1.37	912
61	Maryland, dark loam	L	7.5	"	"	1,000	0.74	740
62	Greenwood	L	7.0	"	"	1,225	2.98	3,650
63	Greenwood, plant cane after soybeans	H	6.4	"	"	875	1.23	1,076
64	L. Texas	L	7.4	"	"	1,150	1.08	1,242
65	L. Texas	H	6.9	"	"	1,075	1.55	1,665
66	Caffery	L	6.2	"	"	1,700	0.81	1,377
67	Caffery	H	5.6	"	"	1,650	1.25	2,062
68	Albania	L	5.6	"	"	1,700	1.78	3,026
69	Albania, poorly drained	L	5.2	"	"	2,100	0.59	1,218
70	Montegut	L	6.2	"	"	1,375	1.50	2,062
71	Montegut	H	7.0	"	"	2,200	0.54	1,188
72	Youngsville	Gl	5.5	"	"	1,675	1.05	1,759
73	Billeaud	Lin.	3.0	"	"	1,100	2.56	2,816
74	Morgan City, Iberia loam	L	7.4	"	"	1,725	1.41	2,433
75	Humus - second sample from Dr. Lutman	-	-	-	"	39,600	-	-
76	Shirley, Gouseaux's plots	Ya	7.6	6/13/46	6/22/46	1,475	3.45	5,089
77	Meeker, Gouseaux's plots, plant cane	Ya	7.5	"	"	1,050	3.81	4,000
78	J. Lanier, C.P. 29/120	L	6.1	6/20/46	"	1,850	1.54	2,849

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
79	J. Lanier, C.P. 34/120, plant cane	H	5.7	6/20/46	6/22/46	725	3.40	2,465
80	Cinclare, plant cane - test plots, Yazoo	L	5.7	"	"	1,775	1.49	2,645
81	Cinclare, Gouaux's plots, plant cane	H	5.9	"	"	1,250	0.66	825
82	Glenwood, C.P. 29/120, stubble	L	6.8	"	"	1,550	1.98	3,069
83	Glenwood, C.P. 34/120, stubble	H	6.1	"	"	775	2.22	1,720
84	Glenwood, plant cane - test plots	L	5.7	"	"	1,625	1.25	2,031
85	Reserve, Gouaux's plots, Yazoo	L	5.7	7/2/46	7/10/46	1,250	2.89	3,612
86	Reserve, Gouaux's plots - north field	H	6.4	"	"	1,225	2.17	2,658
87	Reserve, west side, C.P. 29/120, stubble	H	6.2	"	"	850	0.56	476
88	Cutoff, muck soil	-	4.9	7/8/46	7/27/46	4,337	1.81	7,850
89	Calhoun, N.W. Exp. Sta. - cotton wilt soil	-	6.1	7/19/46	"	2,500	3.24	8,100
90	LSU - check, fall 1945	L	6.1	7/22/46	"	2,033	1.34	2,724
91	LSU - Gr-2-8, sprinkled on planted cane, 52°C, 1945	L	6.3	"	"	2,275	0.50	1,138
92	LSU - check, fall 1945	L	6.2	"	"	2,050	0.47	964
93	LSU - check, 52°C, fall 1945	L	6.6	"	"	2,067	0.71	1,468
94	LSU - Gr-2-8, sprinkled on planted cane 1945	L	6.4	"	"	3,266	1.61	5,258
95	LSU, sampled before adding Act. Gr-2-8	L	6.4	7/29/46	8/9/46	1,375	0.73	1,004
96	LSU, sampled before adding Act. 40-5	L	6.4	"	"	2,050	1.10	2,255
97	LSU, sampled before adding Act. 42-2	L	6.4	"	"	2,675	0.00	0
98	LSU, sampled before adding Act. 52-20	L	6.5	"	"	2,150	0.73	1,570
99	LSU, sampled before adding Act. check	L	6.7	"	"	2,275	0.62	1,410
100	Youngsville, stubble cane	Ol	5.6	8/14/46	8/20/46	1,125	4.46	5,018
101	Billeaud, Gouaux's plots, plant cane	Lin.	4.6	"	"	375	0.25	94
102	Morgan City, Iberia loam	L	7.7	"	"	825	1.64	1,353
103	Albania, Co. 290, plant cane	L	7.0	"	"	1,175	2.64	3,102
104	Caffery, Gouaux's plots, plant cane	L	6.0	"	"	2,300	1.71	3,933
105	Caffery	H	5.8	"	"	1,175	0.16	188
106	Maryland, light colored silt loam	L	5.8	"	"	1,550	1.98	3,069
107	Maryland, dark loam	L	7.2	"	"	1,325	1.32	1,749

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date soil.	Date plated	1000/ gram	Anti. Index	Anti. value
108	Glenwood	L	7.4	8/14/46	8/20/46	1,875	3.16	5,925
109	Glenwood	H	6.8	"	"	1,550	1.14	1,767
110	Montegut	L	6.1	"	"	2,500	2.17	5,425
111	Montegut	H	7.5	"	"	2,375	0.58	1,378
112	Montegut, low black soil, not Sharkey clay	H	7.7	"	"	1,775	3.74	6,638
113	L. Texas, medium heavy	H	7.2	"	"	1,425	4.84	6,897
114	L. Texas	L	6.6	"	"	1,225	3.30	4,042
115	L. Texas	H	6.4	"	"	1,525	2.60	3,965
116	Cinclare, Gouaux's plots	L	6.2	"	"	1,800	2.02	3,636
117	Cinclare, Gouaux's plots	H	6.1	"	"	2,850	1.19	3,392
118	Greenwood	L	8.1	"	"	650	5.00	3,250
119	Greenwood	H	6.3	"	"	825	3.12	2,574
120	J. Lanier	L	6.0	"	"	1,350	1.08	1,458
121	J. Lanier	H	6.0	"	"	1,875	1.26	2,362
122	Reserve, Gouaux's plots	L	6.7	8/16/46	"	1,125	3.50	3,938
123	Reserve, near railroad on plantation road	H	6.3	"	"	2,100	2.13	4,473
124	Shirley, C.P. 34/120	Ya	7.3	8/21/46	8/22/46	2,000	2.44	4,880
125	Meeker, Gouaux's plots, plant cane	Ya	8.1	"	"	1,200	5.32	6,384
126	Melrose, cotton field	Ya	7.7	"	"	1,150	2.18	2,507
127	Cinclare	L	6.5	1/29/47	2/1/47	1,600	2.28	3,648
128	Cinclare	H	6.4	"	"	1,700	1.69	2,873
129	Cora Texas, Filterpress Mud applied fall 1945	FPM	6.2	"	"	2,400	0.86	2,064
130	Glenwood	L	7.0	"	"	3,300	2.66	6,118
131	Glenwood	H	6.4	"	"	2,175	1.31	2,849
132	J. Lanier, C.P. 29/320, stubble	L	7.8	"	"	700	2.03	1,421
133	J. Lanier, C.P. 34/120, stubble	H	6.6	"	"	2,025	1.41	2,855
134	L. Texas	L	7.4	"	"	1,700	3.75	6,375
135	L. Texas	H	6.6	"	"	1,525	1.62	2,470
136	Greenwood	L	7.5	"	"	2,450	2.40	5,880
137	Greenwood	H	6.6	"	"	2,525	2.09	5,277

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
138	Morgan City, Iberia loam	L	6.2	1/29/47	2/1/47	2,425	0.78	1,930
139	Caffery	L	6.4	"	"	3,575	1.12	4,004
140	Caffery	H	6.5	"	"	1,175	1.02	1,198
141	Albania	L	6.5	"	"	1,550	1.06	1,643
142	Billeaud	Lin.	5.3	"	"	1,225	1.02	1,250
143	Youngsville, Filterpress Mud 1946	Ol	5.2	"	"	625	0.35	219
144	Youngsville, around stubble, 4-5" deep	Ol	5.2	"	"	1,375	0.91	1,251
145	Youngsville, from beneath stubble, plow-furrow	Ol	5.0	"	"	200	1.83	336
146	Youngsville, north field, plant cane	Ol	5.6	"	"	2,000	1.31	2,620
147	Shirley	Ya	6.5	"	"	733	2.62	1,920
148	Meeker	Ya	7.9	"	"	700	2.59	1,813
149	Reserve	L	6.5	2/6/47	2/26/47	2,425	1.86	4,510
150	Reserve	H	6.8	"	"	800	2.27	1,816
151	LSU - Act. Gr-2-8 around cane seed pieces 1945	L	6.6	2/11/47	"	2,475	1.31	3,242
152	LSU - check 1945	L	6.6	"	"	2,275	1.47	3,344
153	LSU - Act. Gr-2-8 in soybean middles summer 1946	L	6.6	"	"	2,925	2.65	7,605
154	LSU - check, broth in soybean middles 1946	L	6.5	"	"	3,025	2.74	8,288
155	LSU - Act. 52-20 sprinkled on planted cane 1946	H	7.7	"	"	2,117	1.05	2,223
156	LSU - check, broth sprinkled on planted cane 1946	H	7.6	"	"	2,450	0.45	2,328
157	LSU - Act. + flour dusted on seed cane 1946	H	7.2	"	"	1,186	0.60	712
158	LSU - Act. + sand dusted on seed cane 1946	H	7.0	"	"	1,917	2.16	4,141
159	LSU[2] - Sterilized greenhouse potted soil	L	6.8	2/22/47	"	13	0.0	0
160	LSU - Unsterilized greenhouse potted soil	L	7.2	"	"	2,867	2.37	6,321
161	LSU (SB1)[3] - Sterilized + Act. 119-11	L		3/3/47	3/4/47	0	0.0	0
162	LSU (SB2) - Sterilized + Act. 40-5	L		"	"	0	0.0	0
163	LSU (SB3) - Sterilized + Act. 52-20	L		"	"	0	0.0	0

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
164	LSU (SB4) - sterilized + Act. Gr-2-8	L		3/3/47	3/4/47	0	0.0	0
165	LSU (B1) - Unsterilized + Act. 119-11	L		"	"	2,200	3.94	8,668
166	LSU (B2) - Unsterilized + Act. 40-5	L		"	"	3,300	5.23	17,259
167	LSU (B3) - Unsterilized + Act. 52-20	L		"	"	1,800	2.83	5,094
168	LSU (B4) - Unsterilized + Act. Gr-2-8	L		"	"	1,660	3.37	5,594
169	LSU (A) - Unsterilized - no Actinomyces	L		"	"	1,660	1.22	2,025
170	LSU (SAP) - Sterilized, no Actinomyces, + Pythium	L		"	"	0	0.0	0
171	LSU - Sterilized + Act. Gr-2-8	L		4/21/47	4/22/47	233	7.00	1,631
172	LSU - Sterilized + Act. 52-20	L		"	"	486	6.90	3,353
173	LSU - Sterilized + Act. 100-32	L		"	"	114	18.00	2,052
174	LSU - Sterilized + Act. 108-8	L		"	"	3,833	16.74	64,164
175	LSU - Sterilized + Act. 87-5	L		"	"	586	0.0	0
176	LSU - Sterilized + Act. 40-5	L		"	"	500	5.54	2,770
177	LSU - Sterilized + Act. 110-67	L		"	"	529	5.60	2,962
178	LSU - Sterilized + Act. 113-12	L		"	"	11,450	5.49	62,860
179	LSU - Sterilized + Act. 82-37	L		"	"	3,433	6.50	22,314
180	LSU - Sterilized + Act. 42-2	L		"	"	144	3.38	483
181	LSU - Sterilized + Act. 124-1	L		"	"	24,750	4.27	127,082
182	LSU - Sterilized + Act. 119-11	L		"	"	343	5.15	1,766
183	LSU - Sterilized + Act. N-25	L		"	"	243	9.43	2,291
184	LSU - Sterilized, no Actinomyces	L		"	"	229	0.0	0
185	Billeaud, stubble cane	Lin.	5.0	3/31/47	4/20/47	675	1.04	702
186	Youngsville, plant cane	Ol	5.3	"	"	2,900	1.09	3,161
187	Glenwood	L	6.5	4/17/47	"	1,625	1.58	2,568
188	Glenwood	H	6.4	"	"	900	2.16	1,944
189	Shirley	Ya	6.8	"	"	1,925	1.59	3,061
190	Meeker	Ya	7.4	"	"	1,400	1.83	2,562
191	Meeker, a heavier soil, east of highway	Ya	7.8	"	"	2,275	1.02	2,320
192	Crowley, Joe Heinen farm, before flooding	Rice	6.9	"	"	2,400	1.27	3,048
193	Greenwood	L	7.1	4/22/47	5/6/47	3,450	1.83	6,314

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
194	Greenwood	H	6.5	4/22/47	5/6/47	1,400	1.44	2,016
195	L. Texas	L	7.2	"	"	2,500	2.67	6,675
196	L. Texas	H	6.7	"	"	2,775	1.07	2,969
197	Cinclare	L	6.0	"	"	3,075	1.01	3,106
198	Cinclare	H	6.4	"	"	3,025	1.04	3,146
199	Caffery	L	6.0	4/23/47	"	3,525	0.56	1,974
200	Caffery	H	6.4	"	"	1,875	1.85	3,469
201	Albania	L	6.4	"	"	4,375	2.26	9,888
202	Albania, south of road, medium heavy	H	6.4	"	"	2,200	1.31	2,882
203	Reserve	L	7.8	4/24/47	"	2,950	1.36	4,012
204	Reserve	H	6.6	"	"	1,850	1.65	3,052
205	LSU, hill soil - check, stored under opt. conditions	Lin.	5.6	4/30/47	"	6,200	3.33	20,646
206	LSU, hill soil + rice straw at 140 lbs. N per acre	Lin.	6.1	"	"	12,670	4.80	60,816
207	LSU, hill soil + dried blood at 140 lbs. N per acre	Lin.	5.1	"	"	8,167	3.75	30,626
208	LSU, hill soil + NH_4NO_3 at 140 lbs. N per acre	Lin.	5.0	"	"	7,333	1.94	14,226
215	Cinclare	L	7.2	8/5/47	8/8/47	320	0.80	256
216	Cinclare, Sharkey clay, near swamp	H	6.6	"	"	1,000	2.05	2,050
217	Cora Texas, FPM applied 1945, corn and soybeans just harvested	FPM	6.5	"	"	2,200	1.75	3,850
218	Glenwood	L	6.9	"	"	650	8.14	5,291
219	Glenwood, very heavy, from near swamp	H	6.8	"	"	1,400	2.00	2,800
220	J. Lanier	L	7.1	"	"	614	1.94	1,191
221	J. Lanier	H	5.6	"	"	867	1.71	1,483
222	L. Texas, stubble, north of oak near RR	L	5.6	"	"	1,525	0.69	1,052
223	L. Texas	H	6.9	"	"	950	0.46	437
224	L. Texas, severe root rot area, N.E. of oak	L	7.5	"	"	1,420	0.72	1,022
225	Greenwood, second cut behind mill	L	6.9	"	"	2,425	1.46	3,540

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
226	Greenwood, Sharkey clay	H	7.0	8/8/47	8/8/47	467	1.57	733
233	Morgan City, Iberia loam	L	6.2	"	8/15/47	1,029	1.14	1,173
234	Caffery	L	6.5	8/6/47	"	943	1.67	1,575
235	Caffery	H	6.6	"	"	257	0.30	77
236	Albania	L	6.4	"	"	867	1.85	1,604
237	Albania, dark-medium heavy, south of hwy.	H	6.4	"	"	1,133	1.73	1,960
238	Billeaud	Lin.	5.6	"	"	529	1.11	587
239	Youngsville, north field	Cl	5.8	"	"	686	1.77	1,214
240	Youngsville, near old house place	Cl	6.6	"	"	429	1.00	429
241	Reserve	L	7.4	8/8/47	"	950	1.92	1,824
242	Reserve	H	6.4	"	"	1,833	0.52	953
243	Meeker, Gouaux's plots	Ya	6.9	8/14/47	"	471	1.80	848
244	Meeker, west of railroad	Ya	6.8	"	"	886	1.98	1,754
245	Bubenzer, near Shirley road - hwy. junction	Ya	6.7	"	"	1,100	1.89	2,079
245A	Bubenzer, cane roots sifted from soil 245	-	-	"	"	36,800	2.10	77,280
246	Shirley, near homestead, now in cotton	Ya	6.3	"	"	614	1.90	1,167
247	LSU (check) - Act. added to soybean middles	L	6.9	8/15/47	"	614	0.93	571
248	LSU - Act. Gr-2-8 added to soybean middles 1946	L	6.9	"	"	657	1.29	848
249	Reserve (soil 241) on Pythium agar	L	-	8/8/47	"	1,500	1.69	2,535
250	Meeker (soil 243) on Pythium agar	Ya	-	8/14/47	"	550	4.29	2,831
251	Shirley (soil 246) on Pythium agar	Ya	-	"	"	1,100	1.85	2,035
295	L. Texas, just west of gas tank	L	7.0	10/24/47	11/4/47	1,500	1.79	2,685
296	L. Texas, cane roots from soil 295	-	-	"	"	4,143	1.74	7,209
297	Caffery, Co. 290, stubble	L	6.3	"	"	2,257	1.54	3,476
298	Caffery, cane roots from soil 297	-	-	"	"	11,048	0.81	8,949
299	Billeaud, east of mill, south of highway	Lin.	5.0	"	"	2,733	2.00	5,466
300	Billeaud, Gouaux's plots, C.P. 43/32	Lin.	5.5	"	"	871	0.62	540
301	Billeaud, cane roots from soil 300	-	-	"	"	10,000	0.79	7,900
302	Billeaud, Co. 290, S.W. of Gouaux's plots	Lin.	5.4	"	"	850	1.74	1,479

Table II (continued)

Soil No.	Plantation, with notes for each sample	Soil type	Soil pH	Date coll.	Date plated	1000/ gram	Anti. Index	Anti. value
303	Billeaud, Co. 290 west of mill, south of hwy.	Lin.	7.2	10/24/47	11/4/47	1,529	0.78	1,193
304	Youngsville, Co. 290, north field	Ol	5.5	"	"	1,200	0.75	900
305	Youngsville, Co. 290, 1947 plant. Clinkers in soil	Ol	6.0	"	"	2,686	2.81	7,548
306	Bubenzer, C.P. 29/320, Shirley road - highway junction	Ya	7.0	10/30/47	"	814	1.49	1,213
307	Bubenzer, cane roots from soil 306	-	-	"	"	23,268	1.37	31,877
308	Shirley, 1947 plant. Across bayou from mill	Ya	8.0	"	"	1,657	1.57	2,601
309	Shirley, behind old homestead	Ya	6.8	"	"	1,143	1.57	1,794
310	Meeker, Gouaux's plots	Ya	6.8	"	"	567	2.03	1,151
311	Meeker, C.P. 29/320, west of railroad	Ya	6.8	"	"	2,480	2.63	6,522
312	Meeker, heavier soil, east of highway	Ya	8.2	"	"	1,072	0.90	965

1. Legend: L = light, H = heavy, M = mixed, Lin. = Lintonia, Ol. = Olivier, FPM = filter press mud added to the soil under field conditions.

2. Soils from 159 to 184 inclusive are fairly light soil from L.S.U. Sugarcane Experiment Station which had received various treatments. They were kept under greenhouse conditions in 6-inch unglazed pots.

3. Treatment numbers: S = sterilised, A = no amendments, no Actinomyces, B = no amendments but with Actinomyces inoculated into the soil. The numbers refer to specific Actinomyces isolates.

reliable. This is borne out by an analysis of variance of the individual plate counts of 135 samples (Table III). This analysis included only those samples of untreated field soil from which the colonies in only four plates were counted. The standard error of single plate counts was found to be 3.85 colonies. The standard error of the difference between any two means of the four plates counted was 2.72 colonies. When expressed as the least significant differences between any two populations as determined, this is equivalent to 535,000 organisms per gram of soil.

The mean population of the 135 samples included in this analysis was 1,634,000 Actinomyces per gram of soil. At dilutions of 1:100,000, this was 16.34 Actinomyces colonies per plate. This value divided by the standard error of a single plate will give 23.4 per cent for the coefficient of variability. This value is rather high and would have been considerably reduced had more than four plates been counted.

Those samples from which more than four plates were counted would give a more reliable population value, but because of the variable number of plates counted, no analysis of the data was undertaken.

Table III. Analysis of Variance of the Actinomyces counts from 135 Samples of Field Soils which had only Four Dilution Plates Counted.

Source	D. F.	Sum of Squares	Variance	F.
Total	539	39,212.31	-	-
Samples	134	33,219.81	247.91	16.74*
Plates	3	37.00	12.33	0.83
Error	402	5,955.50	14.81	-

* Denotes that variation due to this source is highly significant.

Standard error of single plate counts, 3.85 colonies.
Standard error of differences between any two means, 2.72 colonies.
Least significant differences between the means of any two plate counts, 5.35 colonies, or 535,000 Actinomyces per gram of soil.
The mean number of Actinomyces per plate was 16.34. The coefficient of variability, 23.44 per cent.

Comparisons of *Actinomyces* from the Different Soils by Isolation Periods

The antibiotic activity of the *Actinomyces* population was found to vary in different soil types. In order to further study this, the sugarcane soils of Louisiana were divided into five general classes. 1. The light alluvial soils along the Mississippi River and Bayou Lafourche. 2. The heavy soils in this same region. 3. The soils along Bayou Teche. 4. The first terrace soils near Lafayette. 5. The Yahola soils along the Red River.

The light soils of class one are alluvial loams and constitute the soils along the river and bayou. The class two soils are for the most part Sharkey clays of varying degrees of fineness. Class three includes all mineral soils along Bayou Teche; with the exception of a heavy soil from Caffery plantation, all were fairly light in texture. Some soil samples from Albania plantation were from poorly drained areas. The first terrace soils were Lintonia and Olivier fine sand and silt loams, which are in general very acid. All samples from the Red River area were from fine sandy loams with the exception of two, which were a clay loam soil. In general, these Red River soils are characterized by neutral to slightly alkaline reactions.

Isolations from the various soils are grouped into four periods. These samples collected from March 1 to June 1, 1946; from June 1 to September 1, 1946; from January 28 to June 1, 1947; and from June 1 to November 1, 1947. The average population counts, antibiotic indices and antibiotic values are shown in Table IV by soil types for the different periods. The population trends and differences are also shown in Fig. 1. The Red River area was not sampled during the first period.

Table IV. Actinomyces from the Different Soils for Each of the Four Periods.

Soil	Period	No. of samples	1000/ gram	Anti. Index	Anti. Value
Red River	1	0	-	-	-
	2	4	1,431	3.76	5,088
	3	4	1,407	1.93	2,335
	4	10	1,080	1.78	2,009
Average		18	1,306	2.49	3,144
Miss.-Lafayette L	1	15	799	2.12	1,664
	2	12	1,417	2.48	3,368
	3	11	2,253	2.13	4,575
	4	7	1,251	1.33	1,653
Average		45	1,430	2.02	2,815
Miss.-Lafayette H	1	16	698	1.30	970
	2	9	1,486	2.20	2,942
	3	11	1,902	1.61	2,843
	4	6	813	1.38	1,409
Average		42	1,225	1.62	2,041
First Terrace	1	3	1,214	1.45	1,739
	2	2	750	2.36	2,556
	3	6	1,467	0.95	1,534
	4	8	1,103	1.22	1,476
Average		19	1,134	1.50	1,826
Teche	1	15	1,318	1.24	1,547
	2	4	1,369	1.54	2,144
	3	8	2,562	1.25	3,344
	4	6	1,081	1.37	1,644
Average		33	1,582	1.35	2,170

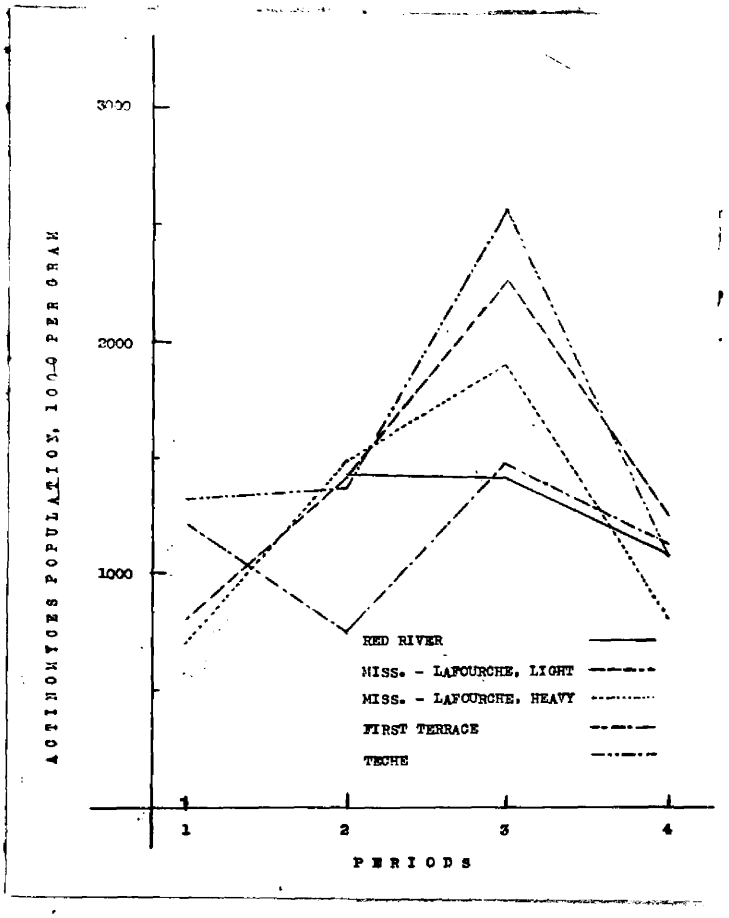

Fig. 1. Trends of the Actinomyces populations in the different soil types.

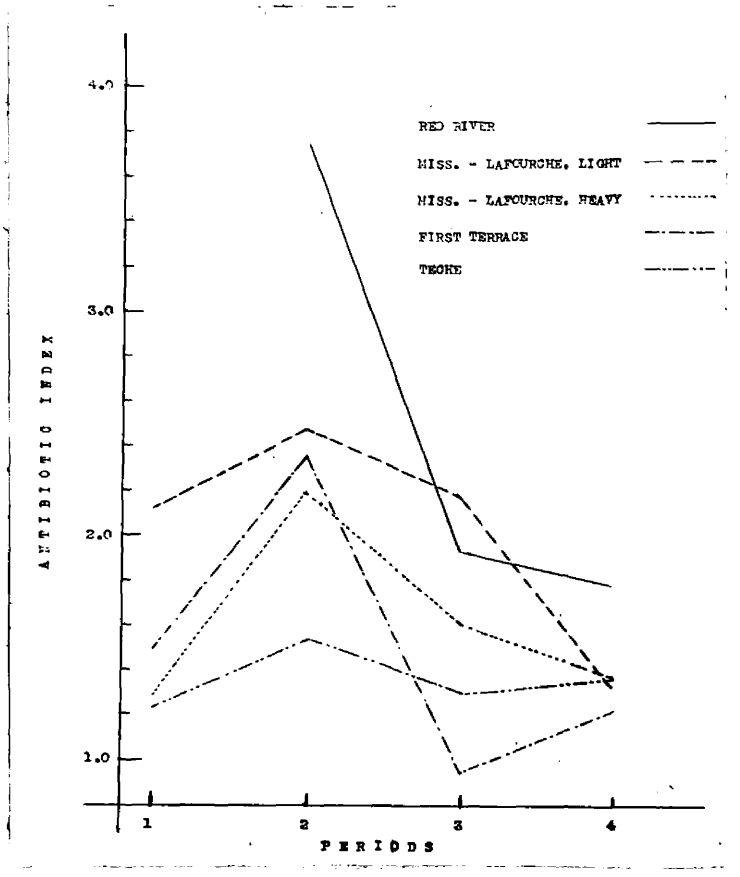

Fig. 2. Trends in the antibiotic index of the Actinomyces in the different soil types.

Three of the soils, the Mississippi River - Lafourche light and heavy, and the Teche, show a common trend in which the Actinomyces population increased from the first through the second and third periods; then there was a marked decrease during the fourth period. The Red River soil showed a marked decrease during the entire period of the study. Counts from samples of this soil taken on different dates varied widely but more or less uniformly. The first-terrace soil showed a more seasonal trend. There was a decrease from spring to summer in the counts for both years. This may have been due to temperature or moisture influence, since both summers were hot and relatively dry in this area.

The antibiotic index trends in the different soils for the different periods are shown in Fig. 2. While the antibiotic index of the Actinomyces from the Red River soils was highest in all the periods, except the third, when it was slightly lower than the Mississippi River - Lafourche light soils, it constantly dropped in activity, very markedly so following the summer determinations of 1946.

The trends are the same for the light soils and the heavy soils of the Mississippi River - Lafourche area, the latter being lower for all periods except the summer of 1947. At this time the average antibiotic index was just a little higher for the heavy soils than for the light soils. Also, during the summer of 1946 the gap between this value for the two soils had narrowed, even though both had increased over the spring determinations.

The antibiotic index of the Actinomyces from the first-terrace soils was the most variable. It was very low during the winter months and increased greatly during the summer months. However, the increase during the hot dry summer of 1947 was not nearly as marked as during

the preceding summer. It should be pointed out that both summers were rather dry for this region. There is a tendency for the results from this area to parallel those from the heavy soils of the Mississippi River - Lafourche area. The average antibiotic values for the Teche soils were lower than the other regions, but were by far the most stable.

The above trends are based on only two years in which the seasons varied widely. The first season was wet throughout the summer. The second summer was unusually cool and wet during the spring and extremely hot and dry during the summer. The variations between two more nearly normal years would probably be much less.

The antibiotic value, by its nature, averages out the differences between the numbers of Actinomycetes and the antibiotic index of the population. That is, it is supposed to indicate the product of factors affecting either the number of Actinomyces or the antibiotic index. Due to the limited duration of the survey and the extreme variation between individual population determinations, it is difficult to draw any conclusions. A study of Fig. 3 reveals that there was a continuous drop in antibiotic value for the Red River soils from about 5,000 in the summer of 1946 to approximately 2,000 during the following summer. The antibiotic values for Mississippi River - Lafourche light soils and the Teche soils tended to parallel each other, with those for the latter somewhat lower during the two intermediate periods. There was a marked increase in the antibiotic value for both soils from the first to the third period, followed by a sharp decline during the fourth period.

The antibiotic values for the Mississippi River - Lafourche heavy soils and the first-terrace soils showed somewhat similar trends. The values increased from the first to the second period, and dropped

considerably in the third, continuing to do so in the fourth, though the decrease was not so great.

In general, the antibiotic value for all soils increased from the first to the second period and decreased thereafter. The most critical time was that between the second period and the third period. During this time the antibiotic value decreased in three soils: the Red River, the Mississippi River - Lafourche heavy and the first-terrace soils. In the other two soils, the Mississippi River - Lafourche light and the Teche, the values increased markedly during this period. The decrease in the antibiotic value for the Mississippi River - Lafourche heavy soil might be attributed to the water-logged condition and poor aeration. The Red River and the first-terrace soils, however, are light in texture and well aerated.

The antibiotic index decreased in all soils during the fourth period (Fig. 3). This would make it appear that factors affecting the population were the cause of the increase in antibiotic values for some of the soils and the decrease for the other soils.

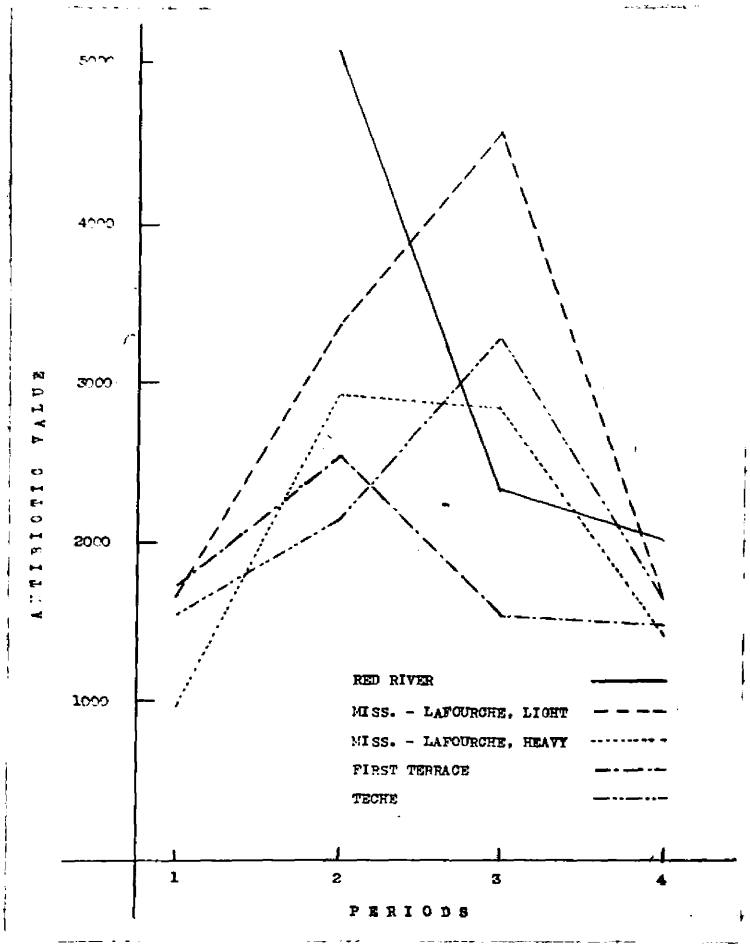

Fig. 3. Trends of the antibiotic values of the Actinomyces for the different soil types.

The Occurrence of Isolates in the Different Inhibition Classes

The occurrence of isolates showing various degrees of inhibition in all soils was rather constant. The data in Table V include results obtained with 8302 isolates from 182 samples of field soil. In order to show their distribution, special classes were set up for those isolates with inhibitory distances greater than 16 mm. This was necessary to determine whether or not these highly antibiotic forms were more common for some soils than for others.

Although the number of isolates in any of these higher inhibition classes was small in comparison to the total number tested, it was somewhat higher from the Glenwood light soil and from the Little Texas heavy soil. There were five isolates from the Glenwood light soil with inhibitory distance greater than 30 mm. These came from three different samples, indicating that they were generally present, even though in small numbers.

The distribution of these antibiotic isolates by soil types has been summarized in Table VI in which the frequency of occurrence in different inhibition classes is shown as percentages. The Red River soil had the highest percentage of antibiotic isolates. However, none of the isolates had inhibitory distances greater than 30 mm., although there was a high percentage of antibiotic isolates in all classes up to 20 mm. inhibition, with a total of 31.76 per cent antibiotic cultures. The next in percentage of antibiotic cultures was the Mississippi River - Lafourche light soils with 28.74 per cent active cultures. The close agreement in the percentage of isolates in the different classes for these two soils corresponded with their average antibiotic indices, which were higher than the other samples.

The Mississippi River - Lafourche heavy soils and the Teche soils also showed a very close similarity in the percentage of isolates in the different inhibition classes. They had 20.45 and 18.51 per cent antibiotic cultures, respectively. The former had an antibiotic index of 1.62, and the latter soil had an antibiotic index of 1.35.

The first terrace soil, with an antibiotic index of only 1.50, had a higher percentage of active isolates than the Teche soil, with 21.87 per cent antibiotic. A very large proportion of these antibiotic isolates were in the 1 - 5 mm. class, with relatively few in the higher inhibition classes. However, there were isolates with all degrees of inhibition up to 30 mm.

From these studies it can be concluded that the Actinomyces in Louisiana cane soils show varying degrees of inhibition. Antibiotic isolates occurred in all the soils included in the survey. The percentages of antibiotic cultures ranged from 31.76 per cent down to 18.51 per cent for the different soil types studied.

Considering all the sugarcane soils studied, including uninoculated soils collected from the Sugar Experiment Station, Baton Rouge, Montegut and the organic soil from the Maryland plantation, a total of 8302 isolates were tested. Of these, 26.40 per cent were antagonistic to P. arrhenomanes. The percentage of all isolates in each of the seven antibiotic classes is shown in Table VI.

The antibiotic index is considered to give a better representation of the antibiotic nature of the Actinomyces population and it is better adapted to calculation. Therefore, it will be used in further discussion.

Table V. The Frequency of Isolates with Different Inhibitory Distances for Plantations and soil types.

	Inhibition Classes - Millimeters								
	0	1-5	6-10	11-15	16-20	21-30	31-40	41-	Total
Alluvial Light Soils									
Cinclare	211	31	25	10		2			279
Glenwood	328	60	37	17	6	1	4	1	454
J. Lanier	237	52	32	16	5		1		343
Little Texas	377	82	35	27	9	2	1		533
Greenwood	200	39	33	16		4			292
Reserve	174	25	25	15	3				242
TOTAL	1527	289	187	101	23	9	6	1	2143
Alluvial Heavy Soils									
Cinclare	269	11	22	7	1	1	1		312
Glenwood	193	28	15	10	3				249
J. Lanier	167	22	15	6	3	2			215
Little Texas	329	53	26	17	3	8	2		438
Greenwood	193	14	13	8	8	1			237
Reserve	207	24	9	9	5	1	1		256
TOTAL	1358	152	100	57	23	13	4		1707
Teche									
Morgan City	193	21	11	5	1				231
Caffery L	423	52	17	17	5	2			516
" H	239	14	10	9	3				275
Maryland L	86	7	7	6			1		107
Albania L	246	34	32	16	4		1		333
" Wet	204	19	14	7	1				245
TOTAL	1391	147	91	60	14	2	2		1707
First Terrace									
Billeaud	312	59	12	8	5	2			398
Youngsville	306	54	17	13	2	1			393
TOTAL	618	113	29	21	7	3			791
Red River									
Shirley	259	41	32	22	5	1			360
Meeker	300	99	48	15	6	3			471
Bubenzer	77	9	11	3	1				101
TOTAL	636	149	91	40	12	4			932
Other Soils									
L.S.U.	387	37	35	11	2	2			474
Montegut	271	19	18	11	10	1			330
Maryland-Black	112	10	8	0	1				131
Degreau & McCall	59	11	11	4	2				87
TOTAL	829	77	72	26	15	3			1022
GRAND TOTAL	6359	927	570	305	94	34	12	1	8302

Table VI. The Percentage of Isolates in each Inhibition Class for each Type of Soil.

Soil type	No. samples	Inhibition Classes - Millimeters							
		0	1-5	6-10	11-15	16-20	21-30	31-40	41-
Yahola	18	68.24	15.99	9.76	4.29	1.28	0.43	0.00	0.00
Alluvial L	45	71.26	13.49	8.73	4.71	1.07	0.42	0.28	0.05
Alluvial H	42	79.55	8.90	5.86	3.34	1.35	0.76	0.23	0.00
First Terrace	18	78.13	14.28	3.67	2.65	0.88	0.38	0.00	0.00
Teche	36	81.49	8.61	5.33	3.51	0.82	0.12	0.12	0.00
Others	22	81.12	7.53	7.04	2.54	1.47	0.29	0.00	0.00
TOTAL		76.60	11.17	6.87	3.67	1.13	0.41	0.14	0.01

A Comparison of Soil Reactions with the Actinomyces Population

The relationship between the soil reaction and the Actinomyces population as shown in Table VII is based upon the mean for all samples with a pH value in the different 0.5 pH ranges. These data are given for each of the four periods as described under the previous section. The averages for all the samples in the different classes are also shown. The variation between the different pH classes are too great to show any trend. However, it should be pointed out that during the first period there is a very definite negative correlation between the pH values and the numbers of Actinomyces per gram.

This relationship, from another viewpoint, is shown in Table VIII. Calculation of the coefficient of correlation for all 24 locations, for the 15 light soils and for the 8 heavy or poorly drained soils, were made. The Sugar Cane Experiment Station soil was not included in calculations of either the light or heavy soils since it is intermediate in type.

There was no significant correlation when all the soils, the light soils only, or the heavy soils only, were considered; however, the heavy soil showed a non-significant negative correlation, that is, heavy soil with an acid reaction tended to have larger Actinomyces counts.

Table VII. A Comparison of Soil Reaction with the Number and Antibiotic Index of *Actinomyces* of Individual Samples for each of the Four Periods of the Study.

pH	Characteristic	Periods 1	2	3	4	Averages
5.25	No. samples	4	1	5	4	14
	1000/gram	1279	375	1360	1414	1107
	Antibiotic index	1.53	0.25	0.88	1.28	0.98
5.75	No. samples	7	10	3	4	24
	1000/gram	1194	1475	2867	1192	1682
	Antibiotic index	1.29	1.74	0.99	1.85	1.47
6.25	No. samples	11	19	18	8	56
	1000/gram	921	1405	2121	1360	1452
	Antibiotic index	1.10	1.55	1.72	1.51	1.47
6.75	No. samples	22	8	10	18	58
	1000/gram	951	1527	2040	1093	1403
	Antibiotic index	1.70	2.10	1.60	1.88	1.82
7.25	No. samples	9	6	6	5	26
	1000/gram	883	1675	2033	967	1390
	Antibiotic index	1.64	2.69	2.42	1.23	2.00
7.75	No. samples	2	6	4	2	14
	1000/gram	550	1179	2094	1364	1297
	Antibiotic index	0.70	3.66	1.48	1.24	1.77
Aver.	1000/gram	963	1273	2086	1232	1388
	Antibiotic index	1.33	2.00	1.52	1.50	1.58

Table VIII. Comparison of the Average Soil Reaction with the Number of Million *Actinomyces* per gram and the Antibiotic Index for the Different Soils Sampled.

Location	Light			Heavy		
	pH	M/gram	Anti. index	pH	M/gram	Anti. index
Ginclare	6.4	1.5	1.5	6.4	1.6	0.8
Glenwood	6.8	1.5	2.0	6.8	1.0	1.5
J. Lanier	6.8	0.9	2.3	6.3	0.9	1.6
L. Texas	7.0	1.3	1.9	6.8	1.3	2.1
Greenwood	7.3	1.8	2.6	6.6	1.3	1.6
Reserve	7.0	1.7	2.3	6.4	1.4	1.6
Albania	6.4	1.5	1.8	6.0	1.7	1.1
Caffery	6.3	2.2	1.4	6.3	1.1	0.9
Morgan City	7.0	1.3	1.3			
Maryland	5.6	1.0	1.5			
Billeaud	5.1	1.1	1.2			
Youngsville	5.6	1.3	1.2			
Meeker	7.0	1.1	2.5			
Shirley	7.5	1.4	2.2			
Bubenser	6.8	1.0	1.7			
L.S.U.	6.6	2.1	1.0			

Correlation coefficient

between pH and - + 0.195 + 0794** - -0.402 + 0.616*

Correlation coefficient

for all samples - + 0.116 + 0.655**

* significant
** highly significant

The correlation between the soil reaction and the antibiotic index was calculated for 193 samples as shown in Table VII. The average for each for these periods was determined and plotted against the different pH values to show differences between the relationship during the four periods (Fig. 4).

The antibiotic index for the second period shows a very marked increase with increase in the soil reaction. The third period shows a similar, though less marked increase, up to the second highest pH range, above which there was a decrease in antibiotic index. The first and third periods show no correlation between soil reaction and antibiotic index.

The general trend for the entire survey shows a general although insignificant increase (9) in the antibiotic index with increase in soil reaction up to pH 7.5, above which there is a decrease (Table VII).

These relationships were also studied by comparing the average antibiotic index and pH value for all samples collected from any given location. The coefficient of correlation was calculated for 24 such locations and found to be highly significant (Table VIII). The correlation of these values for 15 light or well aerated soils were also highly significant. However, it was only significant for 8 heavy or poorly drained soils.

These data indicate that soil reaction is correlated with the antibiotic index of the Actinomyces population, although it does not appreciably affect the size of the population under the ranges included in this survey. The more alkaline soils tend to have the higher antibiotic values.

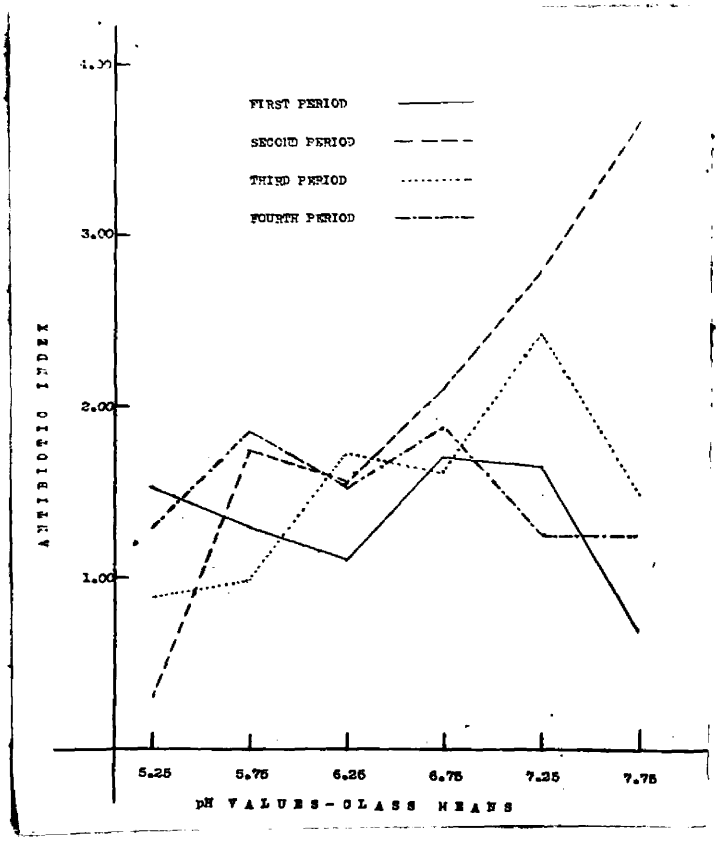

Fig. 4. The effect of soil pH upon the antibiotic index. Based upon average values for each period.

Table IX. Analysis of Variance of the Average Antibiotic Index for the Different pH Values and the Four Different Periods.

Source	DF	Sum of Squares	Variance	F	For sig.
Total	23	10.8028	-	-	-
Periods	3	1.4999	0.5000	1.115	.329
pH value	5	2.5783	0.5157	1.150	.290
Error	15	6.7246	0.4483	-	-

Although comparisons between any two samples must be made with reservation, the following should be brought to attention. A sample was taken from a field at Youngsville plantation on October 24, 1946, which had clinkers in the soil. This indicated that mill ashes had been added to the soil. The date and rate of application was not determined. A comparison of Actinomyces from this field and from an untreated field is made in Table X.

The reaction of this treated soil was not much higher than the untreated; however, the number of Actinomyces and their antibiotic index are considerably higher. Cane was planted in the treated field in the fall of 1947, and in the untreated field in the fall of 1946. Thus, cultivation of the soil in seedbed preparation could have stimulated the development of greater population, and, perhaps the greater antibiotic index also. Therefore, the effect of the ashes upon Actinomyces development needs confirmation, as these data are only suggestive.

Table X. A Comparison of Actinomyces from Soil Receiving Mill Ashes with untreated Soils.

Soil no.	Treatment	pH	1000/gram	Anti. index	Anti. value
304	check	5.5	1,200	0.75	900
305	ashes	6.0	2,686	2.81	7,548

Effect of Rainfall upon Actinomyces

The effect of the amount of rainfall preceding the date of sampling was determined by recording the daily rainfall from the United States Weather Bureau Climatological Data for Louisiana (73). These data were from certain weather stations on or near some of the plantations from which samples were collected during the greater part of the survey.

Cinclare and Reserve Plantations have weather stations on them. The records from Franklin were used to represent the rainfall for the Caffery plantation, Jeanerette for Albania plantation, Lafayette for Billeaud's and Youngsville plantations, and Cheneyville for both Shirley and Meeker plantations.

The total rainfall for the first, and for the first and second week preceding the sampling date were compared with the number of Actinomyces per gram and the antibiotic indices (Table XI). The light and heavy soils were considered separately. This was considered essential since the water relations differ so greatly in these two general types.

The coefficient of correlation was calculated for comparisons of one week's total rainfall with number of Actinomyces and with antibiotic index, and for two weeks' rainfall with the same values in both light and heavy soils. There was no correlation between any of the

eight comparisons (Table XII). The wide variation in the amount of rainfall during this short period more than out-weighed any effect that such difference might have upon the Actinomyces during such a short time.

The samples were classified into three groups depending upon the total amount of rainfall during the two week period. These classes were from 0 to 1.0 inch rainfall, from 1.1 to 3.0, and greater than 3 inches. These values were taken to represent dry, normal, and wet periods respectively.

The mean values for the numbers of Actinomyces per gram of soil and the antibiotic index of the samples in each class were determined (Table XIII). There was a very definite trend for the numbers of Actinomyces per gram to increase with the amount of rainfall during the two weeks preceding the sampling.

The number of Actinomyces was greater following the larger amounts of rainfall, with approximately 88 per cent more Actinomyces in the light soil collected following the wet periods than following the dry periods; with 158 per cent more in the heavy soils following the wet periods than following the dry periods. The numbers in the samples collected following the normal periods of rainfall were intermediate.

There were no differences in the antibiotic index of the Actinomyces isolated following the dry, normal, and wet periods.

Table XI. A Comparison of Total Rainfall, for 14 Days Preceding Date of Sampling, with the Million *Actinomyces* per Gram of Soil, and the Antibiotic Index, for Certain Locations.

Month & year	Sinclare					Reserve				
	Rain-fall	Light m/gm	Light A.I.	Heavy m/gm	Heavy A.I.	Rain-fall	Light m/gm	Light A.I.	Heavy m/gm	Heavy A.I.
March, 1946										
April, 1946	0.0	0.8	1.6	0.3	0.2					
May, 1946										
June, 1946	1.2	1.2	1.5	1.2	0.7	3.9*	1.2	2.9	1.0	1.3
Aug., 1946	3.7	1.8	2.0	2.8	1.2	1.5	1.1	3.5	2.1	2.1
Jan., 1947	2.2	1.6	2.3	1.7	1.7	0.4**	2.4	1.9	0.8	2.3
April, 1947	4.9	3.1	1.0	3.2	1.0	5.3	3.0	1.4	1.8	1.6
Aug., 1947	2.1	0.3	0.8	1.0	2.0	3.2	1.0	1.9	1.8	0.5
Oct., 1947										
	Franklin (Caffery					Cheneyville				
March, 1946	7.8	0.9	3.3	0.3	0.5					
April, 1946										
May, 1946	0.9	2.4	0.7	1.0	1.0					
May, 1946	5.5	1.7	0.8	1.6	1.2					
June, 1946						2.9	1.5	3.4	1.0	3.8
Aug., 1946	2.0	2.3	1.7	1.2	0.2	0.2	2.0	2.4	1.2	5.3
Jan., 1947	3.0	3.6	1.1	1.2	1.0	7.4	0.7	2.6	0.7	2.6
April, 1947	5.4	3.5	0.6	1.9	1.8	4.5	1.9	1.6	1.4	1.8
Aug., 1947	1.5	0.9	1.7	0.3	0.3	0.0	0.6	1.9	0.5	1.8
Oct., 1947	2.2	2.3	1.5			0.3	1.1	1.6	0.6	2.0

* Collected July 2, 1946
** Collected Feb. 6, 1947

Table XI (continued)

Month & year	Rain-fall	Jeanerette Albania - L m/gm	Jeanerette Albania - L A.I.	Rain-fall	Lafayette Billeaud m/gm	Lafayette Billeaud A.I.	Lafayette Youngsville m/gm	Lafayette Youngsville A.I.
March, 1946	9.3	0.9	0.7					
April, 1946	0.0	0.7	3.1					
May, 1946	0.9	0.9	1.1	0.2	0.9	0.7		
May, 1946	1.5	1.7	1.8	4.0	1.1	2.6	1.7	1.0
June, 1946								
Aug., 1946	2.1	1.2	2.6	0.4	0.4	0.2	1.1	4.5
Jan., 1947	2.4	1.6	1.1	2.3	1.2	1.0	2.0	1.3
April, 1947	5.6	4.4	2.3	1.8	0.7	1.0	2.9	1.1
Aug., 1947	0.9	0.9	1.8	0.8	0.5	1.1	0.7	1.8
Oct., 1947				0.0	0.9	1.2	1.2	0.8

Table XII. Summary of Correlations between the Amount of Rainfall Preceding Sampling and the Actinomyces population and its Antibiotic Index; Calculated for Light and Heavy Soils on Basis of Individual Samples.

Time total rainfall considered	Coefficient of correlation of rainfall with Light* m/gm	Light* Anti.index	Heavy** m/gm	Heavy** Anti.index
One week	(+).012	(-).085	(+).115	(-).016
Two weeks	(+).244	(-).010	(+).280	(-).003

* 52 pairs compared, thus .273 required for significance at 5% level.
** 18 pairs compared, thus .456 required for significance at 5% level.

Table XIII. A Summary of Comparisons Between the Rainfall for 14 Days Preceding Sampling with the Number of *Actinomyces* and the Antibiotic Index.

Characteristic	Inches Rainfall 0 - 1.0	1.1 - 3.0	3.0 -
		Light soils	
No. of comparisons	19	17	16
Millions per gram	1.04	1.59	1.88
Antibiotic index	1.87	1.89	1.82
		Heavy soils	
No. of comparisons	3	7	8
Millions per gram	0.70	1.24	1.80
Antibiotic index	1.17	1.14	1.14

Filterpress Mud and *Actinomyces*

It has been reported (88) that the addition of filterpress mud to the soil reduced the severity of root rot. Thus a few soil samples were taken from fields which had received filterpress mud. The data from these studies are shown in Table XIV. The filterpress mud had apparently been pumped onto the fields at Cora Texas and at Glenwood plantations. That at Youngsville had been hauled to the field and spread on the surface of the soil as manure would be. The samples from Glenwood and Youngsville and the first two samples from Cora Texas were taken before the filterpress mud had been turned under and incorporated with the soil. The last sample from Cora Texas was taken after a crop of corn and soybeans had been harvested. That is, the filterpress mud had been incorporated with the soil for several months.

No corresponding samples from untreated soil were taken at Cora Texas plantation, but such samples were available for the Glenwood and Youngsville soils. It is noteworthy that there was a marked decrease in the numbers and a slight decrease in the antibiotic index in the spring following the fall application of filterpress mud. However, the series of studies from Cora Texas show a rapid build up of Actinomyces during the second year following the application. Where comparable samples were available there was no appreciable change in soil reaction following the addition of the filterpress mud.

Table XIV. Actinomyces isolated from Soil that had Received Filterpress Mud compared with Those Isolated from Untreated Soils.

Date	Soil no.	Plantation	Treatment	pH	1000/ gram	Anti. index	Anti. value
5/10/46	56	Glenwood	f.p.m. added 1945	6.4	100	1.59	159
"	46	Glenwood	Light soil (ck.)	6.6	1,275	1.73	2,206
1/29/47	143	Youngsville	f.p.m. added 1946	5.2	625	0.35	219
"	144	Youngsville	from around stubble on adjacent block (ck.)	5.2	1,375	0.91	1,251
"	146	Youngsville	North Field(ck.)	5.6	2,000	1.31	2,620
5/10/46	57	Cora Texas	f.p.m. applied 1945	6.3	217	0.54	117
1/29/47	129	Cora Texas	f.p.m. applied 1945	6.2	2,400	0.86	2,064
8/5/47	217	Cora Texas	f.p.m. applied 1945	6.5	2,200	1.75	3,850

Actinomyces Associated with the Sugarcane Roots

One sample of roots collected at Bubenzer plantation in August, 1947, gave a very high root-soil ratio of Actinomyces, with over 33 times as many Actinomyces associated with the roots as in the surrounding soil. The antibiotic index of the Actinomyces from the roots was also somewhat higher, being 2.10 compared to 1.89 for those isolated from the adjacent soil. This resulted in a very high antibiotic value for the rhizosphere.

Further collections of cane roots were made late in October, 1947. The results of these isolations are shown in Table XV. The root-soil Actinomyces population ratio ranged from 2.8 to 28.6. The antibiotic indices for root and adjacent soil were very close for all except a sample from Caffery plantation in which the antibiotic index for the root population was considerably lower than that for the adjacent soil population. It might be pointed out that this involved a different variety of cane.

A comparison of the percentages of organisms in the different inhibition classes is given in Table XVI. This comparison is based upon the total organisms tested from sugarcane roots and from the surrounding soil. Although a slightly higher percentage of the organisms from the roots was antibiotic, a larger proportion of these was in the least active antibiotic group. Due to the variation between individual comparisons, this difference is not considered significant.

Table XV. Comparisons of *Actinomyces* from Cane Roots with Those Isolated from the Surrounding Soil.

Sample no.	Plantation	Variety	Population 1000/ gram	Population root-soil ratio	Antibiotic activity index	Antibiotic activity value
245	Dubenzer	29/320	1,100	-	1.89	2,079
245a	Roots	"	36,800	33.5	2.10	77,280
306	Dubenzer	"	1,500	-	1.49	1,213
307	Roots	"	23,268	28.6	1.38	31,877
295	Little Texas	?	1,500	-	1.79	2,685
296	Roots	?	4,143	2.8	1.74	7,209
297	Caffery	Co. 290*	2,257	-	1.54	3,476
298	Roots	"	11,048	4.9	0.81	8,949
300	Billeaud's	43/32	675	-	0.62	540
301	Roots	"	10,000	11.4	0.79	7,900
Aver.	Soil		1,309	-	1.47	1,999
	Roots		19,052	13.0	1.36	26,643

* Stubble cane.

Table XVI. A Comparison of the Distribution of All *Actinomyces* from the Roots and from the Surrounding Soil in the Different Inhibition Classes.

Source	Percentage in inhibition classes - Millimeters 0	1-5	6-10	11-15	16-	Total tested
Soil	78.5	9.3	8.5	3.0	0.7	270
Roots	74.5	15.4	7.3	1.7	0.9	233

Cane Yields and Actinomyces

The yield of cane in tons per acre may be considered to give a comparable representation of productivity of any given soil under similar climatic conditions. To study the variation in yields in the different soils, as previously described, the yields of three varieties were considered. These varieties were not selected on the basis of susceptibility or resistance to root rot, but rather because they were grown in test plots at various locations in all soil types. The five-year average yields of variety C.P. 34/120 and the two-year average yields of each C.P. 36/105 and C.P. 36/183, were determined by including the plant cane and first stubble yields. Yield data were secured from varietal test plots of the U. S. D. A. Experiment Station and the Louisiana State Experiment Station. These plots were in several locations characteristic of each soil type.

These average yields for each soil type are shown in Table XVII. The number of tests included in each average is also shown. The trends of the yields for the three varieties are very similar for the different soil types. This indicates some definite difference in fertility.

The average yield of these three varieties of sugarcane are compared with the average Actinomyces population, the antibiotic index, and the antibiotic value for each of the five different soil types. There does not appear to be any relationship between the yield of cane and the size of the population. The antibiotic index and the antibiotic value both show a correlation with the yield of sugarcane. That is, the area that produced the greatest yields also has the greatest antibiotic activity in its Actinomyces population, while those areas producing the lower yields had the least antibiotic activity. This would

Table XVII. Sugarcane Yields for Different Varieties in Different Soil Types Compared with the Actinomyces population, Antibiotic Index, and Antibiotic Value.

Soil type	C.P. 35/105* No. tests	C.P. 35/105* Yield	C.P. 36/183 No. tests	C.P. 36/183 Yield	C.P. 34/120 No. tests	C.P. 34/120 Yield	Aver. yield	1000/ gram	Antibiotic Index	Antibiotic Value
Red River	8	34.8	8	33.0	20	37.6	35.1	1,306	2.49	3,144
Miss.-Laf. Light	24	27.8	23	29.6	55	32.0	29.8	1,430	2.02	2,815
Teche	12	24.8	12	26.0	27	25.3	25.4	1,582	1.35	2,170
First terrace	12	23.1	12	25.0	28	26.7	25.0	1,134	1.60	1,826
Miss.-Laf. Heavy	12	21.6	9	20.7	38	22.8	21.7	1,225	1.62	2,041
Average		26.4		26.9		28.9				

* C.P. 36/105 and C.P. 36/183 are two year and C.P. 34/120 five year average yields in tons per acre.

indicate that the higher sugarcane yields and the higher antibiotic activity of the Actinomyces occur in the same soils.

This correlation might have been much greater if the yield of some root-rot susceptible variety had been available for comparison. However, the testing of all the more susceptible varieties had been discontinued several years previously. The yield data for these susceptible varieties of sugarcane are not considered suitable for comparison with the findings of these studies of the antibiotic microflora, since there have been radical changes in the cultural methods during recent years.

There are two plausible explanations for this relationship; either the presence of the antibiotic microflora affects the yield of cane, perhaps by decreasing the development of the root rotting pathogens, or some ecological factor favors the development of both sugarcane yields and the antibiotic Actinomyces. Based upon the concept that nitrogen, phosphorous and potassium and the trace elements are the basis of fertility, the alluvial soils are considered the most fertile soils studied; yet their yields are lower, especially the heavy soils, than the Red River soil. There are several factors, such as water relations, friability, and microbial activity, that may affect the fertility of the soils as measured by productivity. It is suggested that the antibiotic Actinomyces are a factor in the true fertility of Louisiana sugarcane soils.

GREENHOUSE AND LABORATORY STUDIES

Actinomyces and *Pythium* in Sterilized Soil

To test the activity of the *Actinomyces* isolates in sterilized soil, they were added to soil with and without *Pythium*. Corn, variety White Tuxpan, was used as a test plant. The test included thirteen isolates of *Actinomyces*. Six pots of soil received each of these, three of the pots of soil also received *Pythium*, the remaining three served to check the effect of the *Actinomyces*. A check receiving neither *Actinomyces* nor *Pythium* was also used, as well as one receiving only *Pythium*.

The *Actinomyces* and *Pythium* were added to the soil on February 20, 1947. Each pot of soil received 18 cc. of *Actinomyces* suspension or sterile broth and one-half plate of *Pythium* culture or the equivalent amount of sterile agar medium. Each culture was well mixed into the top two inches of the soil with individual pot labels for each treatment. Water was added to the soil immediately following treatment.

Seven days after treatment, ten grains of corn were placed in each pot and covered with a layer of freshly sterilized soil.

Three successive plantings were made in this soil. The three pots of soil receiving the same treatment were well mixed and reapportioned between the three pots before making the next planting.

The heights of these plants were determined after a period of growth and the average height per treatment calculated. These data are shown in Table XVIII.

The average reduction in height of the plants in Pythium infested soil for the first planting was 43.6 per cent. The reduction was considerably less in the second and third plantings, being 11.3 and 4.3 per cent, respectively.

The height of the plants in the soil receiving Actinomyces and Pythium was greater with eleven of the isolates than the check. However, this was true also for the soil receiving the Actinomyces only. That is, all the plants grown in these soils averaged a greater height than those grown in the soil to which the sterile media were added. This would indicate that the increase was due to factors other than the inhibition of Pythium, perhaps ammonification. This necessitates the use of percentage to compare the inhibitory activity of the different isolates. The data are given in Table XIX. Using these values, any isolates which produced a higher percentage of growth in Pythium infested soils than that in comparable soils without Actinomyces are considered to show some degree of control.

In the first planting four isolates, 52-70, 42-2, 124-1, and M-25, gave increased development of plants in the Pythium infested soil when compared to the treatment without any Actinomyces. The differences are rather large for these. In the second planting three of these same isolates were higher than the check, and two (M-25 and 42-2) were higher in the third planting. All four averaged greater than the check for all three plantings. Isolate 100-32 appeared to have little effect during the first planting, but showed very marked increases

Table XVIII. The Height of Corn Plants Grown in Sterilized Soil to Which *Actinomyces* and *Pythium arrhenomanes* had been added.

Isolate	Average height in inches per treatment							
	Plantings 1		2		3		Average	
	Check	Pyth.	Check	Pyth.	Check	Pyth.	Check	Pyth.
Gr-2-8	14.3	7.9	14.5	11.8	11.6	11.0	13.5	10.2
52-20	13.7	9.0	14.0	11.8	12.5	10.8	13.4	10.5
100-32	14.5	8.2	11.6	11.7	11.2	11.8	12.4	10.8
108-8	14.4	7.7	15.0	12.3	12.4	12.0	13.9	10.7
87-5	15.5	7.3	14.8	13.1	11.6	12.4	14.0	10.9
40-5	15.8	6.9	10.8	11.8*	11.7	10.6	12.8	9.8
110-67	15.2	8.1	-	11.2	10.9	9.9	13.0	9.7
113-12	14.9	8.3	12.0	11.9	11.0	10.2	12.6	10.1
82-37	16.8	9.3	14.6*	11.0	11.1	11.5	14.2	10.6
42-2	14.8	9.1	15.3	11.5	11.9	12.0	14.0	10.9
124-1	13.7	8.6	13.5	12.5	13.0	11.8	13.4	11.0
119-11	15.4	8.2	11.6	11.9	11.6	10.4	12.9	10.1
B-25	13.8	10.2	12.0*	11.2	9.9	10.4	11.9	10.6
Check	13.2	7.5	13.3	11.2	11.8	10.9	12.8	9.9
Average	14.7	8.3	13.3	11.8	11.6	11.1	13.2	10.4
%	-	56.4	-	88.7	-	95.7	-	78.8

*Only two pots considered in this average. Others for various reasons not typical.

Table XIX. The Effect of *Actinomyces* upon the Height of Corn Plants Grown in *Pythium* infested soil expressed as percentage of the *Actinomyces* check.

Isolate	Planting 1	Planting 2	Planting 3	Average
Gr-2-8	55.2	81.4	94.8	75.6
52-20	65.7	84.3	86.4	78.4
100-32	56.6	100.9	105.4	87.1
108-8	53.5	82.0	96.8	77.0
87-6	47.1	88.5	106.9	77.9
40-5	43.7	109.3	90.6	76.6
110-67	53.3	-	90.8	74.6
113-12	55.7	99.2	92.7	80.2
82-37	55.4	75.3	103.6	74.6
42-2	61.5	75.2	100.8	77.9
124-1	62.8	92.6	90.8	82.1
119-11	53.2	102.6	89.7	78.3
M-25	73.9	93.3	105.0	89.1
Check	56.8	84.2	92.4	77.3
Average	56.7	89.9	96.2	78.7

during the second and third plantings. Isolates 87-5 and 40-5 appeared to increase Pythium injury in the first planting. However, the corn plants grown in soil with the former isolate were considerably higher in the second and third planting and those with the latter considerably higher in the second planting. Plants grown with both of these isolates averaged higher than those in the check for the three plantings. Plants grown with certain of the other isolates were higher for either the second or third planting. The most noticeable among these were 113-12 in the second planting, and 108-8 and 82-37 in the third planting. The small differences and interchange of positions indicates that height of corn plants may not be a suitable basis for determining Pythium injury. This has been suggested by Meredith and Semeniuk (80) 1946.

In order to determine the development of the Actinomyces under these conditions, dilution plates were poured and isolations made from the Pythium non-infested series. The antibiotic index was determined for the Actinomyces in the different soils to which had been added various antibiotic Actinomyces. These data are given in Tables I and II, sample numbers 171-184. These samples were taken from the pots April 21, just after the third planting was made. Unfortunately, with several of the soils only a few colonies developed on the plates at the dilutions used. This limited the number of isolates available for testing. A summary of these data is shown in Table XX.

Although only a few cultures were tested for some of the soils, others definitely showed that the Actinomyces population might be very great when introduced into sterilized soil. The antibiotic indices as calculated did not show the true average inhibitory distance, since for these soils some of the populations had a high percentage of isolates with inhibition much above 18 mm. which was used as the mean for the highest class. For example, the actual average inhibitory distance for

the soil infested with Actinomyces isolate 108-8 was 37.04 millimeters instead of the calculated antibiotic index of 16.74.

Table XX. Isolations from Actinomyces Infested, Sterilized Soil in which Corn was grown under Greenhouse Conditions.

Isolate	1000/ gram	Total tested	Anti-biotic index	Anti-biotic value	% parent	Relative height of plant
Gr-2-8	223	9	8.11	1,890	0.0	75.6
52-20	486	28	7.14	3,470	10.4	78.4
100-32	114	1	18.00	2,050	100.0	87.1
108-8	3,833	38	16.74	64,164	90.9	77.0
87-5	586	39	0	0	?	77.9
40-5	500	28	5.54	2,770	0.0	76.6
110-67	529	10	5.60	2,962	80.0	74.6
113-12	11,450	43	5.37	61,486	0.0	80.2
82-37	3,433	50	6.34	21,765	10.0	74.6
42-2	143	8	3.38	438	0.0	77.9
124-1	29,750	51	4.27	127,032	7.1	82.1
119-11	343	13	5.15	1,766	0.0	78.3
M-25	243	14	9.43	2,291	7.1	89.1
Check	229	6	0	0	-	77.3

Although there were exceptions, it seemed noteworthy that certain of the soils with the highest antibiotic values also produced the greatest average height of plants for the three successive plantings of corn in Pythium infested soil. The inconsistency here may be further evidence that the height of plants was a poor indication of the severity

of root rot, or that the antibiotic values as determined were not indicative of the true relationship between Pythium and the Actinomyces population.

In order to further test the activity of the Pythium growing in these soils with the Actinomyces, a 50 gram sample of soil was transferred to, and well mixed in, each of two pots of sterilized soil. This study also included transfers from the soil infested with Pythium but not with Actinomyces and from the Pythium and Actinomyces free soil. In addition two pots of soil received a fresh Pythium culture and two were kept as checks. This made a total of 17 treatments.

The soil was treated and White Tuxpan corn seed were planted on April 25. The height of the plants was determined May 23, at which time the soil was replanted to corn. This second crop was thinned to five plants per pot while the plants were still seedlings. Measurements were made June 18. These data are shown in Table XXI.

The results do not agree fully with those of the original series. However, the plants in soils containing isolates 52-20, 100-32, and 124-1 were high in the three plantings of the original series and in the first planting of the sub-inoculated soils. Isolate 40-5, low in the initial test, was relatively high in the sub-inoculation. The soil receiving the sub-inoculations from the uninoculated soil produced higher plants than any other treatment, probably due to complete absence of Pythium and the addition of airborne saprophytes. It is realized that many factors could affect the results. The second planting showed very little injury due to Pythium; however, this would be expected with the high temperatures that prevailed in the greenhouse during this time (45).

Table XXI. Results of Mixing a Small Quantity of *Pythium*-Actinomyces Infested Soil into Freshly Sterilized Soil as Determined by Height of Corn Plants.

Treatment	Average Height	
	1st planting	2nd planting
Gr-2-8	15.1	15.9
52-20	18.0	16.3
100-32	16.1	14.7
108-8	15.0	13.7
87-5	14.6	16.1
40-5	16.4	17.5
110-67	15.8	16.0
113-12	13.9	16.9
82-37	13.8	15.9
42-2	15.6	15.2
124-1	16.2	16.5
119-11	15.6	16.9
N-25	12.9	17.2
Check	20.0	19.0
Pythium	15.0	17.6
No inoculation	15.4	16.8
Fresh Pythium	12.8	18.4

To further this study a single pot of soil, from the original series, that had received each treatment was kept over summer. No plants were grown in them during the summer, but they were watered every 7 - 10 days and kept covered with a cardboard. Corn was planted in these on November 11 to determine the survival of Pythium in the different soils through the summer. After emergence they were thinned to 5 plants per pot. Due to the development of definite nitrogen deficiency symptoms, 625 mg. of ammonium nitrate in a 2.5 per cent solution was added to each pot on December 20. The plants were allowed to develop until January 28, at which time they were measured and carefully removed from the soil. The roots were carefully washed and their condition noted. The plants for each pot were air dried and then weighed on March 24. These data are shown in Table XXII.

The height and weight of the plants varied considerably and cannot be considered too much as there was only one replicate. The plants had been severely stunted before the nitrogen was added, therefore slight differences in fertility would have resulted in considerable difference in growth. It may be pointed out that the check soils showed a very marked injury to the roots and reduced growth in Pythium infested soil.

With the exception of the plants grown in the soil with the 108-8 isolate, Pythium was not found present in the root tissue from any of the soils with an antibiotic value of 2,500 or greater. The occurrence of Pythium in the originally Pythium-free soils was probably due to contamination. This perhaps occurred by way of the saucers in which the pots were kept during the summer to maintain uniform moisture. Eight of the thirteen soils which had received Pythium and Actinomyces failed

to infect corn growing therein. This may indicate that some considerable time is required for the Actinomyces population to have any effect upon the Pythium in soil under the conditions of these studies.

Table XXII. The Effect of Over-Summering upon the Survival of the Pythium in Actinomyces-infested soil.

Treatment	Average height		Total weight		Root condition Rel.amt.*		Root color**		Pythium pres.***	
	Check	Pyth.	Check	Pyth.	Check	Pyth.	Check	Pyth.	Check	Pyth.
Gr-2-8	20.4	27.8	8.4	10.0	3	3	W	W	0	0
52-20	24.8	27.4	9.5	14.2	3	3	LBB	W	0	0
100-32	25.2	25.4	8.8	8.5	3	3	W	W	0	1
108-8	23.4	21.6	5.3	6.0	2	2	LLB	B	0	3
87-5	29.7	21.6	10.4	6.6	3	3	W	LLB	0	1
40-5	19.2	23.6	4.7	15.0	2	3	W	W	1	0
110-67	22.2	27.0	7.7	11.5	2	3	B	W	2	0
113-12	24.6	27.0	8.4	10.6	3	3	W	W	0	0
82-37	22.8	28.4	7.0	12.3	1	3	LB	W	2	0
42-2	28.6	20.2	9.8	5.8	3	1	W	B	0	3
124-1	27.8	23.0	9.1	10.3	3	3	W	LLB	0	0
119-1	23.8	20.0	9.5	6.1	3	2	W	LLB	0	0
H-25	24.2	19.4	10.1	8.6	2	1	W	B	0	3
Check	29.2	21.4	14.4	5.3	3	1	W	B	0	3
Average	24.7	23.8	8.8	9.3	2.6	2.4	-	-	0.4	1.0

* Relative amount of roots. 1 = few with greatly decreased fibrous roots
2 = considered as average for the treatment. Some fibrous roots present
3 = roots numerous

** W = white, LB = light brown, LBB = light brown in bottom of pot, B = brown.

***0 = no Pythium observed. 1 = only a trace of Pythium present. 2 = considerable Pythium present. 3 = numerous Pythium present in tissues.

The Effect of Different Soil Amendments upon the Activity of *Actinomyces*

The effect of different soil amendments on the antibiotic activities of *Actinomyces* was studied under greenhouse conditions. Corn was used as a test plant. Two sterilized series were included, one receiving *Pythium* and the other without the *Pythium*. One unsterilized series was used, to which no *Pythium* was added, as natural infestation was considered sufficient. Four isolates of antibiotic *Actinomyces*, 119-11, 40-5, 52-20, and Gr-2-8, were used. These represented four cultural types. All treatments were run in triplicate. A mixed, or medium light soil was used. The *Pythium* isolate was the same as was used in the laboratory tests.

The following amendments were added to the soil: treatment B, no amendments except the materials added with the inocula; treatment C, wheat flour at the rate of one ton per acre; treatment D, wheat flour at the rate of one ton per acre plus sodium nitrate at the rate of one-half ton per acre; treatment E, sodium nitrate at the rate of one-half ton per acre; treatment F, dry cane trash at the rate of ten tons per acre; treatment G, molasses at the rate of eight and one-half tons per acre.

In the sterilized series, there were 24 pots in each treatment. Each *Actinomyces* isolate was added to six of these pots of soil, and Pythium was added to three of these. In the unsterilized series, fifteen pots of soil were included for each treatment. Three pots of soil of each treatment received inoculum of each *Actinomyces*, and three pots of soil of each treatment served as checks of the effect of the amendments upon the corn.

The Actinomyces were cultured upon a nutrient broth containing 0.5 per cent dextrose. Sterile broth was kept to add to soils which received neither Actinomyces nor Pythium. The Pythium inoculum was grown on Czapek's agar. Blank plates were poured for soils receiving no Pythium.

Each pot of the sterilized and unsterilized soil received 18 cc of Actinomyces suspension or sterile broth, and each pot of sterilized soil received one-half plate of a Pythium culture or one-half plate sterile agar. Neither Pythium nor agar blanks were added to the unsterilized soils.

Each amendment was well mixed into soil, then the various inocula were added and mixed into the soil, precaution being taken to prevent cross-contamination. The soil was well watered immediately following treatment on March 21, 1947. Six days later, ten grains of corn, variety White Tuxpan, were placed in each pot. These were covered with sterilized soil, for the sterilized series, and unsterile soil, for the unsterilized series.

The unsterilized series. Three successive plantings of corn were grown in these soils. The first was planted on February 27, 1947, and the plants were measured on March 28, 1947. The plants were removed from the soil. Soil from the three pots receiving identical treatment was well mixed, returned to the three pots, and replanted immediately. The plants were measured April 29. The soil was again mixed and replanted. Plants of this third treatment were measured May 20, 1947.

The total stand counts for each treatment for each planting are shown in Table XXIII. There are no appreciable differences in the stand counts for any of the treatments, either due to the amendments or to the

different isolates. Such differences as do exist are considered due to normal variation of the germinability of the corn.

Table XXIII. Stand Counts of the Corn Seedlings for Each of the Three Plantings in Unsterilized Soils to Which Various Amendments and One of Four Antibiotic Actinomyces Isolates had been added.

Treatment No.	Amend.	Plng. no.	Actinomyces isolates: Check	119-11	40-5	52-20	Gr-2-8	Average
B	Check	1	26	27	28	28	27	25.2
		2	23	25	26	24	20	23.6
		3	27	25	29	38	26	27.0
C	Flour	1	28	26	24	25	30	26.6
		2	24	25	22	27	27	25.0
		3	26	28	25	29	27	27.0
D	Flour + nitrogen	1	28	28	25	25	29	27.0
		2	25	24	28	23	25	25.0
		3	27	26	30	27	28	27.6
E	Nitrogen	1	28	27	28	24	28	27.0
		2	20	26	28	26	25	25.0
		3	27	28	25	28	26	26.8
F	Cane trash	1	24	26	27	29	25	26.2
		2	25	27	28	23	25	25.6
		3	28	27	27	28	26	27.2
G	Molasses	1	28	25	23	25	28	25.8
		2	27	24	28	24	27	26.0
		3	26	29	27	28	24	26.8
Average		1	27.0	26.5	25.9	26.0	27.8	26.6
		2	24.0	25.2	26.7	24.5	24.8	25.0
		3	26.8	27.2	27.2	28.0	26.2	27.1
Grand Average			25.9	26.3	26.6	26.2	26.3	26.2

The average height of the plants for each treatment for each planting is shown in Table XXIV. The average differences between the heights of the plants in soil to which inoculum of the different isolates had

been added are not appreciable, either for individual plantings or for the entire test. The range of the latter was only 9.8 to 10.2 inches. These differences were somewhat greater for the first planting.

Table XXIV. The Average Height of Corn Plants for each of the Three Plantings in Unsterilized soil to which various Amendments and Four Different Actinomyces isolates had been added.

Treatment No.	Amend.	pl. no.	Actinomyces isolate: Check	119-11	40-5	52-20	Gr-2-8	Average
B	Check	1	8.7*	8.7	8.4	8.2	9.2	8.6
		2	10.3	10.3	10.0	11.2	10.9	10.6
		3	8.1	9.3	8.2	9.1	9.0	8.7
C	Flour	1	8.1	8.8	9.2	9.4	8.0	8.7
		2	12.3	10.8	10.6	11.8	10.7	11.2
		3	9.0	8.6	8.3	9.1	8.9	8.8
D	Flour + nitrogen	1	9.2	10.6	12.0	10.7	10.4	10.6
		2	13.8	13.0	12.7	13.9	13.4	13.4
		3	9.7	10.6	9.6	9.2	9.8	9.8
E	Nitrogen	1	8.2	10.7	10.6	12.2	9.9	10.3
		2	13.5	12.7	12.9	13.1	12.8	13.0
		3	9.9	9.8	9.7	9.8	10.3	9.9
F	Cane trash	1	7.6	8.2	8.8	7.9	8.0	8.1
		2	11.7	10.7	11.3	11.2	10.8	11.1
		3	9.6	9.1	8.9	9.1	9.5	9.2
G	Molasses	1	7.4	8.3	8.3	8.0	7.9	8.0
		2	9.5	10.1	10.7	9.5	9.4	9.8
		3	9.3	9.8	9.8	9.8	9.3	9.6
Averages		1	8.2	9.2	9.6	9.4	8.9	9.06
		2	11.9	11.3	11.4	11.8	11.3	11.54
		3	9.3	9.6	9.1	9.4	9.5	9.18
Grand average			9.8	10.0	10.0	10.2	9.9	9.98

* The average height of the plants grown in three pots in which ten corn grains, White Tuxpan variety, were planted for each of the three plantings. The values are average height in inches.

The average differences due to amendments added to the soil cannot be explained on the basis of nutrition alone, since flour, cane trash, and molasses failed to reduce the plant growth. Both treatments with nitrogen gave greater growth than the check.

Interaction between the different isolates and the amendments is not evident. That is, no specific combination of Actinomyces isolate and amendment appeared to give consistently higher or lower yields.

Sterilized soil series: The same amendments were used in this as in the unsterilized series. Also, the same Actinomyces isolates; however, no uninoculated checks were used. Pythium was added to the soil in one series of the test, the other receiving only sterile medium. This test was run in triplicate.

The average height of the plants in the three pots of each treatment for three successive plantings of corn, White Tuxpan variety, is shown in Table XXV. The soil in the three pots was well mixed between the different plantings. The plants grown in the Pythium infested soil were, on the whole, much shorter than those grown in the non-infested soil. These averaged for the first planting approximately 60 per cent as tall as the plants in the non-infested soils, with a range from 50.9 per cent for the soil receiving sugarcane trash to 70.9 per cent for the soil receiving the flour. The heights of the plants grown in Pythium infested soil expressed as percentages of development in the Actinomyces inoculated, non-infested soil were as follows: isolate 119-11, 63.5 per cent; 52-20, 62 per cent; 40-5, 60.3 per cent; and Gr-2-8, 58.0 per cent. These values are based upon the averages of the plants grown in 18 pots.

The second planting showed much less injury by the Pythium, since the plants grown in infested soil averaged 96.2 per cent as tall as the

plants in non-infested soil. The soil treatment showing the greatest difference of plants grown in Pythium infested and non-infested soil during the first planting produced taller plants in the second planting in infested soil than in non-infested soil. While the soil receiving the flour showed the least differences for the first planting, it showed the greatest difference for the second planting.

The third planting showed even less stunting in Pythium-infested soil than the second planting. In some of the treatments the plants were taller in the infested soil than in the non-infested soil. These treatments were flour and nitrogen, nitrogen, and cane trash. The reduction in size in Pythium infested soil was less than 6 per cent in the other three treatments. These data are shown graphically in Fig. 5.

There was no significant difference in the activity of the four isolates of Actinomyces.

No specific interaction between any specific amendment and any Actinomyces isolate was evident. Thus, it would appear that the four isolates responded in a similar way to the different amendments.

There was some variation in the stands in these pots, since all plants developing from 10 grains were allowed to grow. To study the effect of the number of plants in the pot upon the height of the plant, the correlation coefficient for these two factors was calculated on single pot basis for each of the Pythium infested and the non-infested series for each planting. These values are shown in Table XXVI.

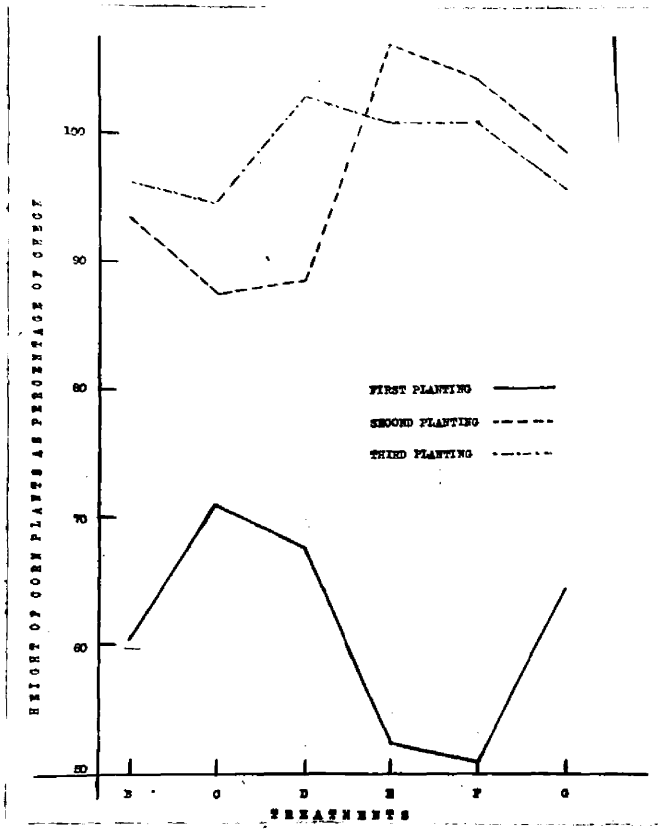

Fig. 5. The effect of different amendments on the relative height of corn plants grown in Pythium infested and non-infested soil. The treatments were as follows: B, check; C, flour; D, flour and sodium nitrate; E, sodium nitrate; F, cane trash; and G, molasses.

Table XXV. The Average Height of Corn Plants for each of the Three Plantings in Sterilized Soil to Which Various Amendments and One of Four Antibiotic *Actinomyces* Isolates had been added. *Pythium* was also added to One Series and the Other Served as a Check.

Treatment No.	Planting	Actinomyces Isolates 119-11 Check	119-11 Pyth.	40-5 Check	40-5 Pyth.	52-20 Check	52-20 Pyth.	Gr-2-8 Check	Gr-2-8 Pyth.	Aver. Check	Aver. Pyth.
B	1	11.3	9.2	13.2	8.0	13.6	7.7	15.3	7.5	13.4	8.1
	2	12.5	13.1	14.1	12.2	12.9	10.7	10.1*	10.9	12.4	11.7
	3	10.3	9.2	11.4	10.6	10.6	10.1*	9.9	11.0**	10.6	10.2
C	1	13.4	8.7	12.8	8.6	12.0	9.8	12.5	8.9	12.7	9.0
	2	13.3	12.4	15.8	12.4	13.6	13.2	14.9	12.6	14.4	12.6
	3	10.5	10.2	10.7	12.4	10.6	10.0	12.8*	10.0*	11.2	10.6
D	1	16.6	9.8	13.9	10.2	14.6	10.8	16.7	10.7	15.4	10.4
	2	15.5	14.0	15.3	11.2	15.3	13.4	12.3	12.9	14.6	12.9
	3	9.4	10.5	11.2	10.6	9.4	11.9	12.8*	10.8*	10.7	11.0
E	1	12.7	7.9	15.0	7.5	16.1	8.0	15.1	7.3	14.7	7.7
	2	11.5	13.6	12.0*	13.8	11.5	13.4	16.7	14.6	12.9	13.8
	3	9.8	10.6	10.5	10.2	10.4*	10.7	13.7	13.3	11.1	11.2
F	1	10.7	5.5	11.0	5.4	10.9	5.4	9.7	5.3	10.6	5.4
	2	12.4	14.3	12.6	12.1	11.2	11.6	11.2	11.2	11.8	12.3
	3	8.3	10.1	10.3	10.3	12.2	11.4	10.6	10.8	10.4	10.6
G	1	9.1	5.8	9.9	5.7	9.0	6.2	9.2*	6.2	9.3	6.0
	2	12.2	14.3	14.1	13.9	13.1	11.9	14.3	12.5	13.4	13.2
	3	10.5	10.5	12.2	11.6	12.4	11.3	11.7	11.3	11.7	11.2
Average	1	12.3	7.8	12.6	7.6	12.7	8.0	13.1	7.6	12.7	7.8
	2	12.9	13.6	14.0	12.6	12.9	12.4	13.2	12.5	13.3	12.8
	3	9.8	10.2	11.0	10.0	10.9	10.9	11.9	11.2	10.9	10.8
Grand Aver.		11.7	10.5	12.6	10.4	12.2	10.4	12.8	10.4	12.3	10.4

Treatments: All soils sterilized by steaming under light pressure for two to three hours. B = no amendments, C = flour, D = flour and nitrogen, E = nitrogen, F = cane trash, and G = molasses.

* One pot missing, therefore the value is the average height of plants in only two pots.

** Two pots missing, therefore the value is the average height of the plants in a single pot.

Table XXVI. The Correlation Between the Number of Plants per Pot and the Average Height of the Corn Plants, and the Regression Coefficient for those with Significant Correlation.

Values	Plantings					
	First		Second		Third	
	Check	Pyth.	Check	Pyth.	Check	Pyth.
Correlation coefficient	.213	.061	(-).969	(-).250	(-).475	(-).593
For significance 5%*	.229	.229	.232	.229	.236	.237
For significance 1%	.298	.298	.302	.298	.307	.309
Regression coefficient	-	-	(-).46	(-).28	(-).52	(-).83

* From G. W. Snedecor, Statistical Methods. Page 149. Iowa State College Press. Ames. 1946.

It is noteworthy that there is no significant correlation in the data from the first planting. This may have been due to the greater availability of nutrients in the freshly sterilized soil, and their subsequent depletion in the second and third planting. There is a significant negative correlation between stand and plant height for Pythium infested and non-infested soils in the last two plantings. However, since the variation in stands was not appreciable when the means of the different treatments were considered, the heights of the plants receiving the different treatments were not corrected.

No dilutions were made from these treated soils to determine the build up of the Actinomyces in the soil. However, a composite sample of all the treatments, taken just before the addition of inoculum, showed complete absence of Actinomyces.

After the plants of the third planting were removed from the soil, one pot was filled with composite soil from three pots receiving the original treatment. Only two of the Actinomyces series were kept, that is, those containing isolates 40-5, and 52-20. These were kept for the unsterilized and the sterilized series containing Pythium. These soils were kept to determine the effect of summer greenhouse conditions upon the survival of Actinomyces and Pythium. No plants were grown in the soil during the summers, but the soils were watered each 7 - 10 days and kept covered with a large cardboard.

Corn was planted November 11, 1947, and thinned to five plants per pot while seedlings. On December 20, 25 cc of a 2.5 per cent ammonium nitrate solution was added to each pot of soil.

The height of each plant was recorded on January 28, 1948. The soil was then removed from the pots and the roots carefully removed from the soil and washed. Examination of the roots showed those grown in inoculated sterilized soil to be healthy, with practically no discoloration of the roots and just an occasional decayed root tip. There were numerous fine laterals present.

The plants grown in unsterilized soil receiving the different treatments, including Actinomyces, were found to have considerably darker roots, with more terminally decayed roots. The roots in the bottom of these pots showed a greater amount of discoloration than in the sterilized soil.

Microscopic observations recorded in the accompanying table show the occurrence of Pythium in the tissues to be correlated with these general observations. Pythium oospores were observed in roots from three of the sterilized series that received Actinomyces, and this only

in an occasional root. They were observed in all roots from all of the unsterilized series except two, and as a rule they were common in discolored and decayed tissue.

This difference in amount of Pythium in the inoculated sterilized and unsterilized series may be due to a more rapid build up of Actinomyces when added to the sterilized soil. It should be pointed out that the Pythium inocula were much higher in the sterilized soil as shown by the severe infection and stunting of the first planting of corn following inoculation. The unsterilized series was only naturally infested.

Differences in height and weight of plants, TableXXVII, are probably due to nutritional factors, since the plants grew under nitrogen deficient conditions for the early part of the experiment.

Table XXVII. Development of Corn in *Pythium* Infested Soil Carried Through the Summer under Greenhouse Conditions.

Treatment[1]	Average height	Average weight	Oospores in roots	Notes
SB	28.8	10.8	No	No decay
SBP	22.2	9.0	Yes	Root tip decay
SBP2	18.8	5.7	Yes	Very little decay
SCP2	19.4	5.1	No	No decay
SDP2	24.4	8.4	No	No decay
SEP2	28.4	9.8	No	No decay
SFP2	25.6	9.1	No	No decay
SGP2	27.0	13.6	No	Roots discolored, not limited to tips
SBP3	20.0	6.6	No	Roots discolored, not limited to tips
SCP3	23.8	8.0	No	
SDP3	21.8	6.6	Yes	Very little tip decay
SEP3	26.2	9.5	No	Very little decay, other fungi
SFP3	25.2	11.6	Yes	Very little tip decay
SGP3	26.2	10.8	No	Some decay, not limited to tip
B2	18.4	4.2	Yes*	Considerable decay
C2	22.8	5.3	Yes*	Very little decay
D2	24.0	7.0	Yes	Very little decay
E2	24.4	6.7	Yes	Very little decay
F2	30.0	9.4	Yes	Considerable decay in bottom of pot
G2	28.0	8.2	Yes	Considerable decay in bottom of pot
B3	18.6	5.9	Yes*	Considerable decay in bottom of pot
C3	22.4	7.4	Yes	
D3	26.0	6.5	Yes*	Very little decay - typical
E3	23.4	4.8	Yes	
F3	21.0	6.3	No	
G3	20.8	5.9	No	Considerable discoloration, non-septate fungi present.

*Isolated the *Pythium* from these plants. This is all from which isolation was attempted.

1. Key to treatments: S = sterilized, B = no amendments, C = flour added, D = flour and nitrate added, E = nitrate added, F = cane trash added, G = molasses added, P = infested with *Pythium*, 2 = received *Actinomyces* isolate 40-5, 3 = received *Actinomyces* isolate 52-20.

Isolations from Field Soils to Which Antibiotic Actinomyces Had Been Added

The 1945 treatments: The first Actinomyces were added to field soils in the fall of 1945. Soil samples 90 - 94 were taken July 22, 1946. (Tables I and II). So far as the Actinomyces are concerned there were only two treatments: with Actinomyces, and check. Although these were applied to hot-water treated and non-hot-water treated canes. The inoculations were made by sprinkling a suspension of Actinomyces isolate Gr-2-8 on the seed pieces after they were placed along the furrow.

The differences in the number of Actinomyces isolated per gram, the antibiotic index, and the antibiotic values are rather small. None of the isolates tested from the treated plots were significantly higher than those from the checks (Table II).

A second sampling from these plots was made on February 11, 1947. These samples were taken from directly around the old seed pieces where the inoculum had been added. Results of isolation from these samples are shown in Tables I and II, samples 151 and 152. A single isolate, 151-52, from the treated soil shows inhibition and cultural characteristics similar to Gr-2-8, the isolate placed in the soil.

The results of isolations from these two samplings, expressed as averages, are shown in Table XXVIII. Although these studies have been very limited, the treated soils were somewhat improved in all respects.

Table XXVIII. The Results of Isolations from Field Soils to Which Antibiotic Actinomyces were added during 1945.

Treatment	No. samples	1000/ gram	Antibiotic index	Antibiotic value
Check	4	2106	1.00	2,125
Gr-2-8	3	2672	1.14	3,213

The 1946 tests: Three different inoculation tests were set up during the fall of 1946. Test 1. The Actinomyces were added in suspension to soybean middles just before they were turned under in the late summer. When these beds were opened for planting the cane was supposedly placed in the soil containing the Actinomyces. Test 2. As in the 1945 test, the Actinomyces in suspension were sprinkled on the planted cane just before covering. Test 3. Three Actinomyces isolates, grown individually, were filtered from this cultural medium on the same filter paper. Then they were well mixed into wheat flour. This was dusted at the rate of 150 pounds per acre onto the seed pieces and adjacent soil. As a check treatment the Actinomyces were mixed with sand and applied to the seed pieces.

Isolations were made from these areas. The results are shown in Tables I and II as samples 153 to 158 inclusive, and 247 and 248. The first series of samples was taken February 11, 1947 and the second August 15, 1947. These samples were taken from around the seed pieces where the inoculum had been placed.

A comparison of the results obtained in the treated and check of each pair of samples will show very little difference (Table XXIX). Notable exceptions were in the case of the Actinomyces with flour and the Actinomyces with sand. The latter gave higher counts, with an

antibiotic index of 2.16 compared to 0.60 for the inoculum with flour. Although based upon a single sample, it would appear that flour at 150 pounds per acre is not conducive to the development of antibiotic Actinomyces.

There was no difference in the proportion of isolates in the higher inhibition classes for the treated and untreated soils. However, there were more representatives in the higher inhibition classes in the Actinomyces - sand treatment than in the Actinomyces - flour treatment.

Table XXIX. Isolations from Field Soil to Which Antibiotic Actinomyces were added during the Fall of 1946.

Test	Soil no.	Treatment	1000/ gram	Anti. index	Anti. value
1	154	Check	3025	2.74	8288
	153	Gr-2-8	2925	2.65	7605
	247	Check	614	0.93	571
	248	Gr-2-8	657	1.29	848
2	156	Check	2450	0.95	2328
	155	52-20	2117	1.05	2223
3	158	Act. and sand	1917	2.16	4141
	157	Act. and flour	1186	0.60	712

Soil Cross-Inoculation Studies

Since Actinomyces in different soils varied in number and in degree of antagonism toward Pythium arrhenomanes, tests were set up to determine the effect of mass transfers of soil microflora from some of these different soils into different sterilized soils. This inoculation

was accomplished by thoroughly mixing a rounded tablespoonful, approximately 50 grams, of unsterilized soil with each pot of sterile soil.

In this test only two soils, Glenwood light and the Shirley Yahola light soil, were included. The following treatments were included in this test: 1. Unsterilized; 2. Sterilized soil infested with Pythium just before planting; 3. Sterilized check; 4. Sterilized, recontaminated with soil from the same location; and 5. Sterilized soil recontaminated with soil from the other location. The soil-inocula were added one week before the corn was planted. The test was run in triplicate.

Ten grains of White Tuxpan corn were planted in the soil in each pot on May 5. The soil was watered at suitable intervals. The height of the plants was determined 24 days after planting. The average height of the plants in each treatment is shown in Table XXX.

Table XXX. The Number and Average Height of Corn Plants for First Planting in Cross-Inoculation Studies.

Treatment	Shirley			Glenwood light		
	No. plants	Aver.* height	Color	No. plants	Aver. height	Color
Non-sterilized	23	10.6	Gr.	27	14.9	lt. gr.
Sterile + Pythium	28	9.4	"	24	13.5	" "
Sterile	30	14.8	"	25	14.6	dk. "
Sterile + raw soil from same location	27	15.0	"	28	16.9	green
Sterile + raw soil from other location	30	15.0	"	27	16.2	v. dk. gr.

* Average height in inches.

The Glenwood light soil gave considerably better growth when mixed with either of the soils than when used alone. The differences in the case of the Shirley soil were not nearly as marked. The growth of plants in sterile soil re-infested with Pythium was somewhat less than in the unsterile soil.

To determine the effect of the microflora in the soil over a period of time, the same pots of soil, devoid of plants, were kept watered on the greenhouse bench over summer. On November 2, the soil in each pot was carefully pulverized, and 10 grains of White Tuxpan corn planted therein. Stand counts were made 16 days later, when the plants were thinned to five per pot. The height of these plants was determined on December 14. The plant roots were carefully separated from the soil and washed. The plants from each pot were air dried in the greenhouse, and then weighed. These data are shown in Table XXXI.

Although the differences are small, it is interesting to note that plants growing in both of the cross-inoculated soils were the tallest in each soil series.

Table XXXI. The Average Height and Total Dry Weight in grams of the Second Planting of Corn in the Cross-Inoculation Studies.

Treatment	Shirley soil			Glenwood light soil		
	Emergence	Aver. height	Total weight	Emergence	Aver. height	Total weight
Non-sterile	20	12.8	7.30	20	11.5	3.45*
Sterile + Pythium	21	11.5	7.30	17	11.4	4.00*
Sterile	26	12.8	8.10	20	14.8	8.90
Sterile + raw soil from same location	24	13.4	9.05	25	13.6	9.80
Sterile + raw soil from other location	27	13.9	9.85	23	15.9	9.40

*Plants had been thinned to five for each pot. However, cut worms had infested two pots of each of these treatments, destroying five plants in each treatment.

The Effect of Different Sources of Nitrogen upon the Actinomyces Microflora

The following treatments were set up, using Lintonia fine silt loam from the Horticultural Experiment Station at Baton Rouge. 1. Check, no amendments. 2. Rice plant meal with 0.78 per cent nitrogen. 3. Dried blood, with 11 per cent nitrogen. 4. Ammonium nitrate with 35 per cent nitrogen. These materials were added to the soil at the rate of 140 pounds of nitrogen per acre. These soils were incubated at 28°C. The moisture content was kept near optimum by adding the required amount of water at weekly intervals. The containers, 600 millimeter beakers, were kept covered with watch glasses. Samples were taken for Actinomyces isolations after 53 days incubation. The results of these isolations are shown in Table I, sample numbers 205 through 208 inclusive. The antibiotic nature of the populations is shown in Table II, sample numbers 205 through 208.

Due to the number of cultures developing in each dilution plate, along with some fungal and bacterial colonies, most of the cultures isolated from soil receiving treatment number two became contaminated, and on this account only a few cultures were available for testing. This fact greatly reduced the validity of the antibiotic index determined for this sample. The very large numbers of Actinomyces developing in the untreated soil when held under the conditions of this experiment indicated that the potential Actinomyces populations may be much higher than that normally obtained from field soil.

The following counts were obtained from the soils receiving the different treatments: Rice straw, 12,670,000; Dried blood, 8,670,000; Ammonium nitrate, 7,333,000; Check, 6,200,000, values in Actinomyces per gram of soil. The antibiotic index for three of the treatments was as follows: Dried blood, 3.7; Check, 3.33; and Ammonium nitrate, 1.94.

Although based upon a single determination, the results appear to indicate that the antibiotic index, as well as the number of organisms per gram, may be changed with different soil treatments.

Specificity of Antibiotic Activity

Several Pythium isolates were collected during 1945 and 1946. These were from various hosts and showed some different types of vegetative growth. They were tested with two or three of the Actinomyces isolates which were antibiotic to Pythium arrhenomanes. A single streak of the Actinomyces isolates was made across one side of a petri dish on Czapek's agar. After two days incubation the Pythium inoculum was placed on the opposite side of the plate. The tests were either duplicate or triplicate. The results of these studies are shown in Table XXXII and Figs. 6 and 7.

Table XXXII. Specificity of Antibiotic Activity in Different *Pythium* Isolates.

Pythium isolate*	Pathogenicity	Act. isolate	Complete inhibition** Test I	Complete inhibition** Test II	Type of response of the *Pythium* isolate to inhibition
Pythium arrhenomanes	Very path.	Gr-2-8	27	16	A very few advanced hyphae to the point from which the measurements were made; i.e., aerial inhibition 10 to 20 mm. greater than complete inhibition. These *Pythium* isolates responded alike to each of the *Actinomyces* isolates.
		40-5	24	27	
		113-24	-	27	
G-2	Path.	Gr-2-8	30	-	
		40-5	18	-	
		113-24	-	-	
L-2	Path.	Gr-2-8	28	-	
		40-5	24	-	
		113-24	-	-	
3 1/c	Path.	Gr-2-8	25	-	
		40-5	17	-	
		113-24	-	-	
Lt-13	Weakly	Gr-2-8	29	-	Advanced edge thin for 5 to 10 mm. Then a ring of raised hyphae.
		40-5	22	-	
		113-24	-	-	
"Sesbania"	Weakly	Gr-2-8	0	-	Growth up to the *Actinomyces* colony but not across it.
		40-5	0	-	
		113-24	-	-	
"Squash"	Weakly	Gr-2-8	-	0	Just a slight trace of aerial inhibition.
		40-5	-	0	
		113-24	-	-	
Lt-12	No	Gr-2-8	29	-	Advanced edge of *Pythium* growth thin for 10 to 20 mm., aerial growth thin behind inoculum.
		40-5	22	-	
		113-24	-	-	
P. spinosum	No.	Gr-2-8	16	16	Pythium growth abrupt along the inhibited margin.
		40-5	8	16	
		113-24	-	16	
"Cane leaf"	?	Gr-2-8	-	16	Same as isolate Lt-13.
		40-5	-	20	
		113-24	-	23	
"Cabbage"	?	Gr-2-8	-	0	Grows up to but not across.
		40-5	-	4	Aerial inhibition 15 mm. (Abrupt).
		113-24	-	0	Grows up to but not across.

*The Pythium isolates are indicated by host where identity is unknown. The "G" and "Lt" series were isolated from cane field soil. Isolate 3 1/c is an old unidentified stock culture. *Pythium spinosum* (?) was taken from the lower leaves of a young sugarcane plant.

**The average inhibition given in mm.; Test I, an average of 3 determinations. Test II, an average of 2 determinations with the exception of *P. arrhenomanes*, which had only a single replication in this test.

Fig. 6. The response of different Pythium isolates to two Actinomyces. Pythium isolates from left to right: P. arrhenomanes, P. spinosum, and the Pythium isolated from sesbania seedling. The top Actinomyces is Gr-2-8 and the lower 40-5.

Fig. 7. The response of different Pythium isolates to two Actinomyces. Pythium isolates from left to right: P. arrhenomanes Isolates Lt-13 and Lt-12. The top is Actinomyces Gr-2-8 and the lower 40-5.

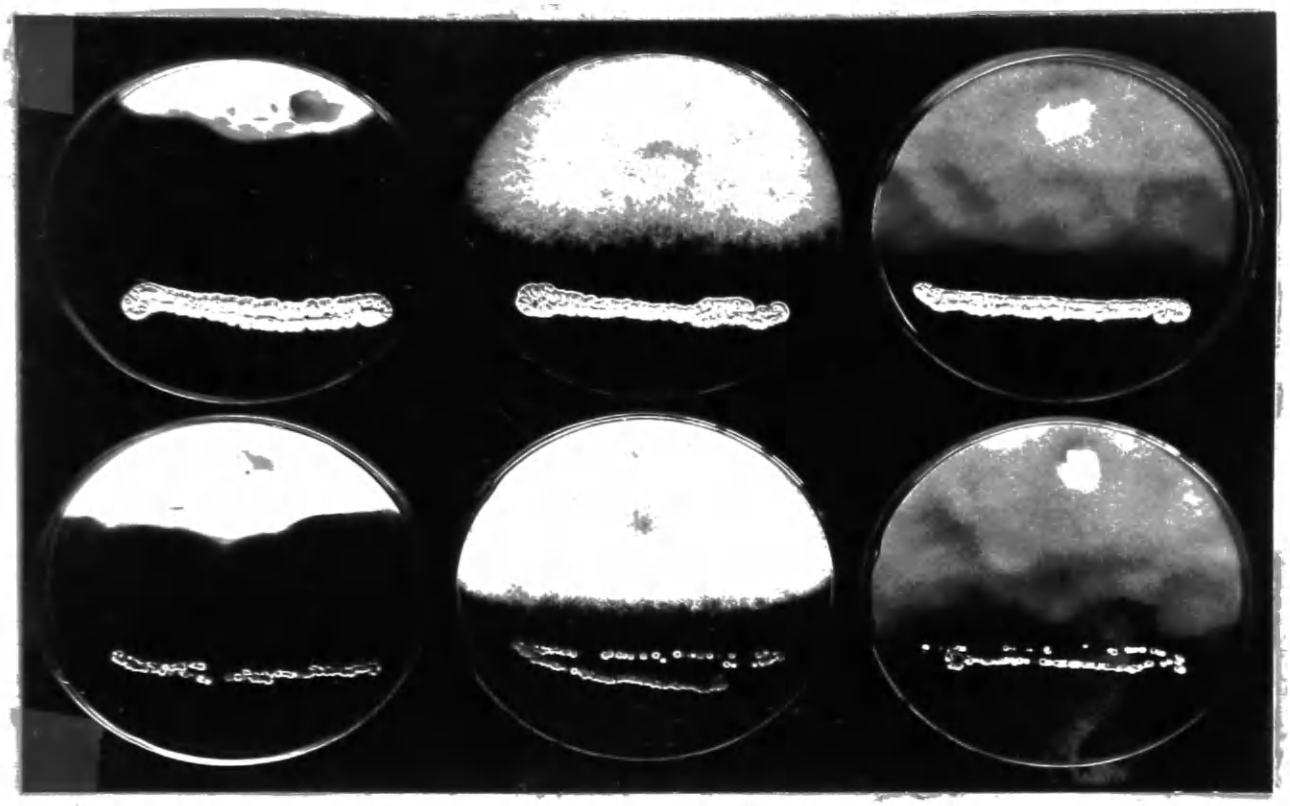

Fig. 6

Fig. 7

From these data it is evident that the different isolates of Pythium were specific in their response to these Actinomyces isolates. The quantitative differences varied somewhat for the different determinations; however, measurements were made from the foremost hyphae and these were often very sparse, with most of the mycelium inhibited some distance back.

The qualitative differences ranged from abrupt inhibition of aerial and subterranean mycelium, as with Pythium spinosum Sawada, to a gradual thinning out, with advanced growth consisting of subterranean mycelial strands and the aerial strands gradually thickening. This type of response was typical of Pythium arrhenomanes grown with Actinomyces isolate Gr-2-8. Also the aerial inhibition may be abrupt although some distance from the advanced edge of growth. This type of response was obtained with the Pythium isolate from cabbage grown with Actinomyces isolate 40-5.

Another definite inhibition response was that of the Pythium isolate Lt-13 grown with Gr-2-8 or 40-5 Actinomyces isolates, and the Pythium isolate from cane leaves with 40-5 or 113-24 Actinomyces isolates. This response was typified by a rather abrupt inhibition of the foremost edge, followed by a line of thick aerial mycelium about one centimeter wide, followed in turn by a narrow zone in which the aerial mycelium was considerably inhibited (Fig. 7).

Using the testing procedure of Edgerton, Tims, and Mills (36), the Pythium isolates, P. arrhenomanes, G-2, L-2, and 3 l/c, were determined as pathogenic on corn roots. The Pythium isolate Lt-13, and those obtained from squash and Sesbania seedling, were mildly pathogenic. Pythium spinosum and Lt-12 were non-pathogenic.

The pathogenic isolates all responded very much alike to the different Actinomyces. The mildly pathogenic isolates gave two different types of inhibitory response. Pythium isolate Lt-13 formed a raised line of aerial mycelium some distance from the foremost edge of the Pythium growth. The other two isolates were not inhibited. The two non-pathogenic isolates, P. spinosum and Lt-12, were inhibited to about the same degree, the former with a thinned-out margin and the latter with an abrupt inhibition of both aerial and subsurface mycelium.

Isolation of *Actinomyces* on a *Pythium*-Nutrient Agar

A selectivity for antibiotic organisms when grown on a substratum containing the organisms to be inhibited has been reported (114). The lysis of an inhibited organism is a common phenomenon with bacteria, especially when the cells have been killed (114). Meredith (79) reported lysis of *Fusarium oxysporium cubense* (E. F. Sm.) Woll. and Rein. by antibiotic *Actinomyces*. The following experiment was set up to check the possibility of selectivity of the *Actinomyces* colonies, and the lysis of dead *Pythium* mycelium.

The *Pythium*-nutrient agar was made up according to the following procedure. *P. arrhenomanes* was grown on nutrient broth containing 0.5 per cent dextrose for 27 days. The mycelium was removed by filtration, and rinsed through several changes of water. The mycelial mat was then pressed dry between filter papers and weighed. It was then emulsified in water in a Waring blender. The medium was made up as follows: four grams of nutrient broth extract, 20 grams of agar, a sufficient amount of the emulsion to be equal to five grams of *Pythium* mycelium, and enough water to bring the volume up to a liter. The resulting medium was highly opaque. The medium was tubed and sterilized for 30 minutes at 15 pounds pressure.

Dilution plates were poured for three soils. Dilutions in Conn's glycerol asparginate agar were used as check. Results of isolation and antibiotic tests are shown in Table XXXIII. There was no evidence of lysis of the mycelial fragments in the 20 days that plates were kept. The size of the *Actinomyces* colonies was much smaller than on Conn's agar and bacterial colonies were more numerous.

Table XXXIII. A Comparison of the Actinomyces which developed on Pythium-Nutrient Agar and on Conn's Glycerol Asparginate Agar.

Location	Conn's agar				Pythium-nutrient agar			
	Sample no.	1000/ gram	Anti. index	Anti. value	Sample no.	1000/ gram	Anti. index	Anti. value
Reserve	241	950	1.92	1,824	249	1,500	1.69	2,535
Meeker	243	471	1.80	848	250	550	4.29	2,831
Shirley	246	614	1.90	1,167	251	1,100	1.85	2,035
Average	-	678	1.87	1,280	-	1,050	2.61	2,467

These data show consistently higher counts on the Pythium-nutrient agar. The antibiotic indices are variable, a fact probably due to the limited number of isolates tested, averaging 26 for each sample. The Actinomyces from two of the soil samples had somewhat higher indices when isolated on Conn's agar. The Actinomyces from the other sample had an antibiotic index over twice as great as the isolates from Pythium-nutrient agar. The average antibiotic value for the isolates which developed on Pythium-nutrient agar was somewhat higher than the value for those which developed on Conn's agar.

The Effect of Depth of Medium on Inhibition

To determine the effect of the depth of the medium on the degree of inhibition (85), the amount of medium added to each petri dish was varied. The following amounts of Czapek's agar were poured into 9 cm. petri dishes: 10, 20, and 30 cc. A single Actinomyces isolate was transferred to each plate. These were then incubated for two days at 28°C. before the Pythium was added to the opposite side of the plate. The test was run in triplicate. Measurement of the inhibited distance

was made after incubation for seven days. Both the maximum and the minimum inhibition were recorded in millimeters for each plate.

The results as shown in Table XXXIV give the average maximum and minimum distances of inhibition in millimeters. There was very little variation in the inhibition of the Pythium for the different depths of medium (Fig. 8). The maximum inhibition was not as constant as the minimum. The latter has been used in all other studies.

Table XXXIV. The Effect of the Depth of Medium upon the Distance at which the Pythium is inhibited.

Isolate number	Average inhibition* - 3 replications					
	10 cc		20 cc.		30 cc.	
	Max.	Min.	Max.	Min.	Max.	Min.
119-11	5	2	5	0	5	0
52-20	55	38	56	39	58	40
Gr-2-8	55	38	49	37	49	36
40-5	47	22	41	26	38	26
Average	40.2	25.0	37.8	25.5	37.5	25.5

* Expressed as millimeters inhibition.

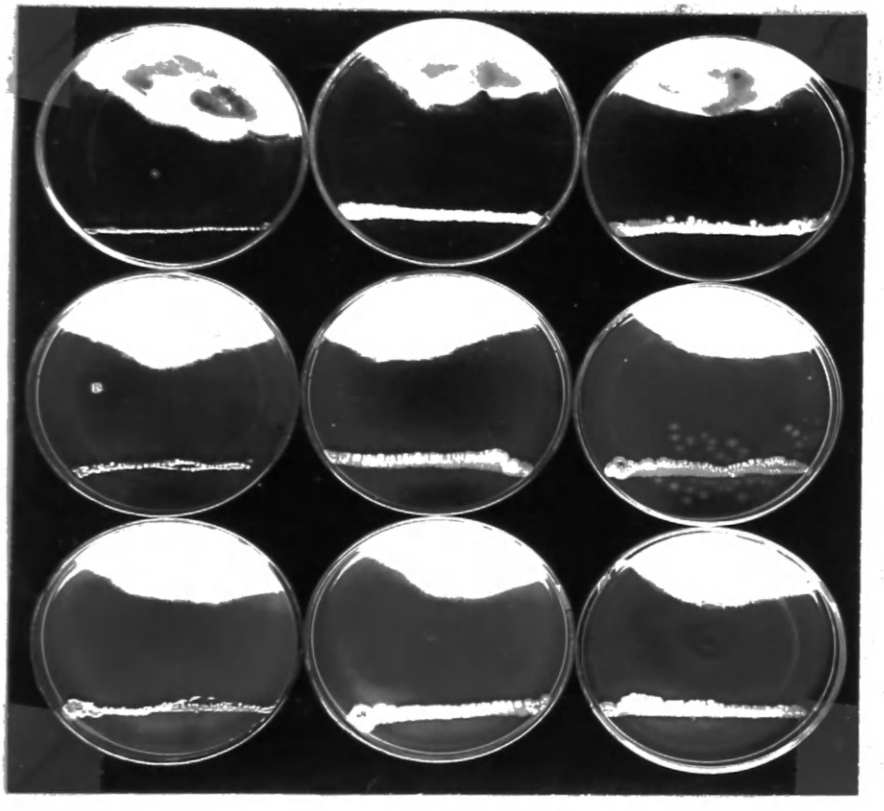

Fig. 8. The effect of the depth of medium upon the inhibitory distances. From top to bottom: 10, 20, and 30 cc. medium per plate; and from left to right: Actinomyces isolates 40-5, Gr-2-8, and 52-20.

DISCUSSION

During recent years considerable attention has been given to the inhibition of soil-borne plant pathogens by saprophytic microorganisms. Although the exact part that these antibiotic organisms have in limiting pathogenic organisms in the soil under natural conditions is still uncertain, the distribution and antibiotic activity of Actinomyces have been considered as possible ecological factors in the etiology of sugarcane root rot. Early phases of this work have been reported (25, 26).

This study of the Actinomyces in the sugarcane soils of Louisiana has shown that they were widely distributed. The total numbers were not influenced by soil pH within the ranges of the soils studied. The average size of the population did not vary appreciably in the five soil types considered. However, the population apparently was influenced by the rainfall.

The occurrence of antibiotic isolates varied from 18.51 per cent to 31.46 per cent for the five different soil types. The percentage of antibiotic isolates was closely correlated with the average inhibitory distance, that is, the antibiotic index. However, the relative frequency of the occurrence of isolates showing the different degrees of inhibition was not always correlated with the antibiotic index.

The antibiotic index of the Actinomyces population was not appreciably sensitive to the amount of rainfall, but showed a marked positive correlation with the soil reaction. It also varied widely for the five different soil types. These relationships between the antibiotic

Actinomyces and their environment would indicate that the relative proportion of antibiotic Actinomyces might be increased by altering certain soil factors. This was indicated in laboratory studies in which dried blood or rice straw added to Lintonia fine sandy loam not only resulted in an increased Actinomyces population but also increased the antibiotic index. The same amount of nitrogen added to the soil in the form of ammonium nitrate was less active in this respect.

Limited studies of the Actinomyces associated with the cane roots show that they were several times more numerous on the roots than in the adjacent soil. The antibiotic index of the Actinomyces associated with the roots was approximately the same as that for those in the surrounding soil; that is, there was no apparent selectivity of the roots for the antibiotic or non-antibiotic Actinomyces.

The large numbers of Actinomyces associated with the cane roots would greatly increase the total antibiotic activity, that is, the antibiotic value, in the immediate vicinity of the roots. The effectiveness of the antibiotic activity of the rhizosphere (101) in reducing the root tip injury by P. arrhenomanes will depend upon the age of the root when the Actinomyces population increases on it. If the increase of the population occurs before the lateral roots are formed, they might be protected until growth was well initiated. The counts as determined in these studies would have included any Actinomyces associated with the disintegrating cortex (102) of the old roots.

The average yield of three varieties of sugarcane in test plots in each of the five soil types was closely correlated with the antibiotic activity of the Actinomyces. This correlation might be explained by one of two hypotheses. Either the presence of the antibiotic Actinomyces favored the development of sugarcane, possibly by the reduction

of root rot, or both the antibiotic Actinomyces and the sugarcane are favored by the same environmental and soil conditions.

Limited isolations failed to show any increase of the antibiotic isolates when they were added to field soils. However, there was a very rapid increase when certain of these antibiotic isolates were added to sterilized soil under greenhouse conditions.

The results of studies concerning the control of Pythium root rot of corn by the addition of antibiotic Actinomyces to sterilized Pythium-infested soil, showed no significant control when the reduction in the height of the Pythium infected plants was considered as a criterion of root rot injury. However, there were no infected roots on corn plants grown in soil several months after the Pythium and Actinomyces were added. This suggests that the Pythium was no longer present or had lost its virulence.

Several isolates of Pythium from different sources were grown with some of the more antibiotic Actinomyces in laboratory culture. Various types of inhibition responses occurred. This suggests that the different Pythium species are specific in their response to the antibiotic activity of the Actinomyces. All Pythium isolates which produced typical root rot symptoms on corn responded alike to the Actinomyces.

SUMMARY

Variation in the severity of sugarcane root rot has not been satisfactorily explained on the basis of nutritional and environmental factors. The non-pathogenic soil microflora has been considered as a possible factor in the etiology of the root rot disease. The distribution and antibiotic activity of Actinomyces are considered as an ecological factor in Louisiana sugarcane soils.

The total Actinomyces population for each soil sample was determined by use of a standardized dilution procedure. The antibiotic activity of individual Actinomyces isolates against Pythium arrhenomanes was determined for a number of isolates from each soil sample.

A total of 182 samples of untreated field soil, taken at different times during the two year period, were studied. From these 8302 Actinomyces isolates were tested in culture for antibiotic activity against P. arrhenomanes.

Although the Actinomyces population varied widely between individual samples, it was correlated with the amount of rainfall preceding the time of sampling. It was not correlated with the seasons or soil reaction within the pH range of 5.5 - 7.5. The counts did not differ appreciably in the five different types of soil studied.

The occurrence of antibiotic isolates varied from 18.51 to 31.46 per cent for the five different soils. The average of all samples was 23.40 per cent. The percentage of isolates tested showing the various degrees of inhibition were as follows: no inhibition, 76.60;

1 - 5 mm. inhibition, 11.17; 6 - 10 mm. inhibition, 6.87; 11 - 15 mm. inhibition, 3.67; 16 - 20 mm. inhibition, 1.13; 21 - 30 mm. inhibition, 0.41; 31 - 40 mm. inhibition, 0.14; and above 41 mm. inhibition, only 0.01 per cent. In general, there was close agreement between the percentage of antibiotic isolates and the average inhibition of antibiotic index.

The antibiotic index was not affected by the amount of rainfall, but did show a marked positive correlation with the soil reaction up to pH 7.5; above this level there was a slight decrease. The antibiotic index varied in the five different soil types.

The average yield of three cane varieties grown in the five soil types was compared with the Actinomyces population and the antibiotic activity. There was no correlation between the yields and the total population, but the antibiotic index was significantly correlated with the yields. The antibiotic value is the number of thousands of Actinomyces per gram soil multiplied by the antibiotic index. The antibiotic value showed a high degree of correlation with the yields.

The Actinomyces were found to be from 2.8 to 33.4 times as numerous on the cane root than in the surrounding soil. There were no appreciable differences in the antibiotic activity of the Actinomyces associated with the roots in comparison to those in the adjacent soil. The possible significance of this highly antibiotic population in the Rhizosphere in the etiology of root rot is discussed.

The antibiotic index and the Actinomyces population were higher in a soil which had received mill ashes than in the untreated soil.

The addition of filterpress mud to canefields resulted in a temporary decrease in the Actinomyces population. After about one year there was an increase in the counts.

Various isolates tested in the greenhouse for their antibiotic activity in the soil failed to show appreciable disease control of the first planting of corn. However, in succeeding plants there was evidence that the Pythium was less active in the soils with Actinomyces. Isolations from specially treated soils indicated a rapid increase of certain of the introduced isolates.

Under the conditions of these studies none of the several soil amendments appeared to influence the activity of the Actinomyces in either sterilized or unsterilized soil.

Isolations from soils in the cane field to which had been added an antibiotic isolate failed to show any appreciable increase of the introduced isolate.

Cross-inoculation of soils to determine the influence of different soil microflora upon the productivity of a given soil failed to show any marked difference in the two soils used. However, the sterilized recontaminated soils gave better growth than either the unsterilized or the sterilized soils.

Different species and isolates of Pythium did not react in the same way to specific antibiotic isolates of Actinomyces. All Pythium isolates tested that produced typical symptoms on corn roots did respond alike to the different Actinomyces included in this study.

Equal amounts of nitrogen from three different sources were added to a field soil. Limited isolations indicated that addition of rice straw and dried blood resulted in larger Actinomyces populations than did the addition of ammonium nitrate. The antibiotic index was also somewhat higher for the Actinomyces grown with the organic sources of nitrogen.

Soil dilutions were plated out in a medium containing a suspension of Pythium mycelium. Although more colonies developed on this medium than on Conn's agar, growth was slow. There was no lysis of the dead mycelium around the Actinomyces colonies.

The depth of the medium did not affect the degree of inhibition of the isolates used in comparative studies.

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BIOGRAPHY

William Earl Cooper was born on December 25, 1917 in Bradley County, Arkansas. He graduated from the New Edinburg High School in 1936. He was employed by the Southern Lumber Company of Warren, Arkansas, as timber cruiser from November, 1936, until January, 1938.

He entered Arkansas A. and M. College in May, 1938, and received his Bachelor of Science degree in Botany in May, 1942. Meanwhile, he served as Observer in Charge of an Off-Airways Weather Station located on the campus, taught Science in Drew Central High School the fall semester of 1941, and conducted outlying field tests of tomato culture during the spring of 1942. From graduation until February, 1943, he was an Assistant to the Dean of Men at Arkansas A. and M. College.

In February, 1943, he went to the Arkansas Fruit and Truck Branch Experiment Station as a Scientific Assistant. From September, 1944, until June, 1945, he was on leave of absence to do graduate work at Oklahoma A. and M. College. He received his Master of Science degree in Botany and Plant Pathology from that institution in July, 1945. The title of his thesis was Top Necrosis - A Virus Disease of Guar. He resigned from the Arkansas Experiment Station September, 1945, to continue his graduate studies at Louisiana State University as a Graduate Research Assistant. He is now a candidate for the Doctor of Philosophy degree in May, 1948.

EXAMINATION AND THESIS REPORT

Candidate: William E. Cooper

Major Field: Botany and Agronomy

Title of Thesis: The Occurrence of Antibiotic Actinomyces in the Sugarcane Soils of Louisiana and Their Effect Upon Pythium Root Rot

Approved:

C. W. Edgerton

Major Professor and Chairman

W. E. Jeroaye

Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

April 26, 1948.