BIOLOGICAL STUDIES OF THE RED-BROWN BUTT ROT FUNGUS, POLYPORUS SCHWEINITZII FR.

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BIOLOGICAL STUDIES OF THE RED-BROWN BUTT ROT FUNGUS, POLYPORUS SCHWEINITZII FR.

by

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I. Acknowledgements

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II. Introduction

The accent on reforestation during the past twenty years and especially the establishment of conifer plantations, show promise of development of epiphytotics in the future that heretofore have not been encountered. Polyporus schweinitzii Fr. has already manifest itself as a cause of disease in young conifer plantations (50). The purpose of this study is an attempt to collect the pertinent information in the literature and to obtain further knowledge concerning the growth of this species.

The Reed Pinetum on the campus of Michigan State College was the site used for most of the field work covered in this study. This white pine plantation, consisting of three and one-half acres, was planted in the spring of 1896 with five year old trees. It is the oldest white pine plantation in the state of Michigan (10). The present study was carried out during the fiftieth year of the stand. Thus stumps left from the fifty-year thinning were available for a study of the incidence of the disease.

The history of the Pinetum is relatively complete and numerous studies on soil type, site indices, and growth increments are available and have proven to be of invaluable aid in this study.

III. Review of Literature

The velvet-top fungus, Polyporus schweinitzii Fr., is the cause of red-brown butt rot of conifers. This rot is sometimes referred to as brown-cubical butt and root rot (2). It has also been called red-spruce disease (31).

Red-brown butt rot has long been considered a major silvicultural and pathological problem in the Northwest forest region of the United States. Boyce (5) considers it the most serious butt-rot of conifers in the United States, both as to the number of trees attacked and the volume of wood destroyed. Besides the specific loss of wood in the butt log, infected trees are also subject to windthrow.

Emphasis has been placed on this root and butt-rot in the Eastern United States only during the past twenty years.

Until the work of York, Wean, and Childs (51), Polyporus schweinitzii was observed only on older trees. Weir (43) lists Polyporus schweinitzii as one of the most important wood-destroying fungi of jack pine (Pinus banksiana) but mentions that it does not produce appreciable decay until the tree reaches a period of decline. This age is placed at approximately sixty to eighty years. Earlier reports in the literature lead one to believe that Polyporus schweinitzii is a fungus

which affects mature and overmature trees. However, York, Mean, and Childs (51) observed seriously damaged eastern white pine (Pinus strobus L.) and red pine (Pinus resinosa Ait.) under twenty five years of age in upper New York state. Mean (46) was able to artificially produce infection in seedlings and in two year old nursery stock.

The modes of infection and transmission have given rise to various and sundry hypotheses. Boyce (5) states that infection is largely through basal wounds, particularly fire scars, and that the incidence of butt rot will be high in coniferous fire-scarred stands. He mentions that it has been assumed that the fungus spreads through the soil to infect roots and that infection may also occur by contact of a decayed root with a healthy one. Cartwright and Findlay (7) believe that infection always takes place through the roots. Hiley (24) suggests that the fungus enters by the roots and spreads upward in the stem. Boyce, after Hiley and contemporaneously with Cartwright and Findlay in 1938, concluded that the theory of root entrance and soil spread of the fungus needed substantiation. Very little actually was known about the mode of infection.

Mean (46) proved that infection of seedlings and two year old nursery stock of Pinus strobus L. would take place through the roots by placing needle-duff inoculated media directly in contact with the root

systems. Hunting and Chapman (27) concur insofar as infection through basal wounds is concerned, but contend that cases of apparent root entry were observed. They further state that insects were commonly associated with the decay where it had originated at basal wounds.

Boyce (5) discussed a particular case of infection characterized by a cluster of sporophores issuing from an old knot fifty one feet above the ground on a living Douglas fir (Pseudotsuga taxifolia (Poir) Britt.) with a diameter at breast height of sixty inches. There was no decay at the butt. Therefore, he concluded that the decay was the result of local infection, probably through an old knot from which sporophores issued. Although this is an odd and specific case, it shows definite evidence of possible means of infection.

Various factors which influence susceptibility have also been discussed extensively. Peace (34) discusses the following factors as influencing the presence and severity of infection for the butt-rots in general:

1. previous crop
2. elevation, slope and aspect
3. soil
4. drainage
5. rainfall
6. vegetation
7. composition of stand
8. method of establishment
9. age
10. density
11. rate of growth
12. distribution

Baxter (2) believes that water table fluctuations have a bearing on susceptibility. York (50), in discussing root, root-crown, and butt fungus parasites,

states that the manner of placing the roots in the soil at time of transplanting undoubtedly plays a part in susceptibility. It has been generally observed that there is a marked relationship between rate of growth and prevalence of butt-rot in balsam fir (Abies balsamea (L.) Mill.) (33).

Presence of the disease in a stand and the number of diseased stems are difficult to ascertain. Fruiting bodies occur near the ground level, being found on roots and old stumps. They are difficult to locate as they are hidden by soil, leaves, and other organic debris. York, Wenn, and Childs (51) report failure to either locate sporophores or to isolate Polyporus schweinitzii for several years in a stand which was later proved to be heavily infected.

Riley (24) suggest the use of a Pressler increment borer to check the incidence of the disease. However, it is the opinion of many forest pathologists that borings are of little avail in most cases. The incipient decay is usually confined to a small portion of the basal area of the stem and could be too easily missed.

Polyporus schweinitzii has been studied by the forest pathologist from the pathological and silvicultural points of view. Fritz (16) describes the cultural appearance and growth of this fungus on various media together with a cultural key for various species of wood-destroying fungi. Childs (9) gives a descrip-

tion of the variability of the fungus in culture. Hubert (26) discusses effects of the organism on the wood of the host and described microscopic details. Cartwright and Findlay (7) described the sporophore of Polyporus schweinitzii and included a resume of the occurence, characteristics of the rot, cultural and physiological data, and the economic importance of the disease.

IV. Hosts

An examination of the literature revealed that a complete host list for Polyporus schweinitzii Fr. was not available. Since such a list would be of value to pathologists, a tabulation of host species and genera has been assembled in Table 1.

In addition to the species of higher plants subject to attack by Polyporus schweinitzii, Bourdot and Galzin (4) reported that cherry is frequently attacked. They also find the organism capable of parasitizing hazel.

Rankin (40) makes special note that red-brown butt rot of conifers is one of the few wood rots which occurs in arbor-vitae (Thuja). Overholts (36) makes the statement that this organism attacks all genera of conifers except Juniperus. Of the genera of the order Coniferales that are represented in the United States,

Sequoia, Taxodium, Cupressus, and Juniperus have not been found as hosts in the literature.

The list presented in Table 1 attests to the rarity of the disease on hardwoods. The specimen collected on Quercus alba was reported by Overholts (37) on the roots of a living tree near Moorestown, Pennsylvania in an open, mature stand consisting entirely of oak and hickory species. There is no indication that conifers have ever been present in the woodlot.

Of the host species listed, Douglas fir, pine and spruce are the common hosts in the United States (6).

V. Synonomy

Polyporus schweinitzii has been placed in numerous genera and renamed a number of times. This has probably been due to its variability, its variety of hosts, and its wide distribution. The names Polyporus schweinitzii Fr. and Phaeolus sistotremoides (A. and S.) Murrill are the most common in the literature. However the following names have been cited as synonyms:

Boletus sistotremoides Alb. and Schw. Consp. Fungi. 243 1805.
Polyporus schweinitzii Fr. Syst. Myc. 1:351. 1821.
Dædalea ellisii Lenz. Schwamme 62. 1831.
Polyporus spectabilis Fr. Nov. Symb. 43 1851.
Polyporus schweinitzii var. maximus Fr. Cooke et Quelet Olavis Hymenomycetum. 178.
Polyporus hispidoides Peck. New York State Museum Reports 23:21. 1870.
Polystictus Schweinitzii (Fr.) Karst Enumeratio Boletinearum; Revue Mycologique 13:18. 1881.

Cladoderis Schweinitzii Quel. Ench. Fungi: 169 1886.
Coltosporus sistotremoides (Alb. et Schw.) Murr. Krypt. Fl. Schles. I:[illegible]. 1893.
Fomitella sistotremoides (Alb. et Schw.) Murr. Polyporaceae of North America; Bull. Torrey Bot. Club 31:339. 1904.
Placodus sistotremoides (A. and S.) Murr. Bull. Torrey Bot. Club 32:597. 1905.
Polyporus sistotremoides (Alb. and Schw.) Lefr Nouv. III. [illegible] 1905.

In addition to this list, Fries (16) has noted Boletus sistotrema Alb. et Schw., Daedalea sordida Wahlb., and Sistotrema sordidum Swartz. In another publication (17), he has listed Boletus sinuosiformis Dicks, Boletus pellucidus Pth., Boletus carolinus Lour., and Polyporus lentiformis Fries. Murrill (32) questions the synonomy of Polyporus sinuosus Fr. The following synonyms have also appeared in the literature but are not commonly found; Inodermus Quel., Rhacodus Schweinitzii Fr. (Prb.)., Irpex Schweinitzii Fuck., and Polyporus Schweinitzii Fr. var. hispidoides Pk.

Murrill (33) gives a partial explanation to the synonomy. He states that the irregular pores caused the transfer from Polyporus to Daedalea. The likeness to Polystictus perennis caused the transfer to Polystictus. Then, overlooking this relationship and largely on account of its hyaline spores, it was classified in the genus Cladoderis.

The type of Polyporus tabulaeformis was an old plant of Polyporus schweinitzii sent to Berkeley. A specimen similar to it from North Carolina was sent to

Fries and received the name Polyporus spectabilis. Because of its resemblance to Polyporus biscidus, Peck separated the sessile form of his species found on tree trunks and named it Polyporus biscidoides.

Later, Murrill (34) states that he did not realize that the genus Rhaeolus had been raised from subgeneric rank previous to the time he assigned the generic name Romellia. He related Rhaeolus and Polyporus as two different genera of the family Polyporaceae, Rhaeolus having sporophores with a brown context and Polyporus having sporophores with a white context. Schroeter (43) separated Cobrosporus and Polyporus in a manner similar to Murrill's Rhaeolus and Polyporus.

Cooke and Quelet (11) recognize Polyporus schweinitzii but also include the variety Polyporus Schweinitzii var. maximus Fr. Hartig (20) uses the name Polyporus mollis but admits that he had made an incorrect identification and concedes Polyporus schweinitzii to be the correct name. It should be noted that Fries left the "t" out of Schweinitzii in his original description of the fungus.

VI Cultures

The following sources of material were used for the cultural work:[1]

Culture	USDA accession number	Original source
2	731	Black cherry, Pennsylvania
6	F1[illegible]7	[illegible]
10	[illegible]7-S	Pinus strobus, West Virginia, 1934
14	7111[illegible]	[illegible]dock, Ottawa National Forest, 1936
1[illegible]	7101[illegible]-S	Black spruce, Nicolet National Forest 193[illegible]
22	71707-S	Norway pine, Duluth Minn. 1937
26	[illegible]6853	Pine, Rock Hill, South Carolina 193[illegible]
30	[illegible]4[illegible]-R	Betula lutea. New York 19[illegible]
34	7[illegible]-R	Pine, California 19[illegible]
38	71[illegible]-S	White pine, Manistee National Forest 193[illegible]
46		Pinus strobus, East Lansing, Michigan
50		Pinus strobus, East Lansing, Michigan

All isolates, with the exception of two, cultures No. 18 and 34 produced sporophore tissue in vitro. Sporophores were small for the most part. Sporophores of culture 26 most closely resembled natural fruiting bodies. Sporophore formation followed the descriptions and interpretations of Childs (2) very closely except that 83.3 of the cultures grown showed sporophore tissue as compared with 28.6 of Childs' cultures.

VII pH

It was found desirable to obtain the optima, minima,

[1]The cultures, with the exceptions of the local isolates, were supplied through the courtesy of Dr. Ross W. Davidson from the Bureau of Forest Pathology collection at Beltsville, Maryland.

and maxima pH of some of the cultures being worked with in the course of these studies in order to eliminate the effect of pH as an unknown variable.

Malt agar was prepared according to the following formula:

25 gr. malt extract
17 gr. Bacto agar
1000 cc. distilled water

The malt agar was autoclaved for fifteen minutes at fifteen pounds pressure. The acidity was adjusted by the use of dilute lactic acid and dilute sodium hydroxide. Gross changes in pH were determined by use of Enochemia Ltd. indicator paper. A Beckman pH meter was used for more accurate pH tests. In order that the temperature adjustment on the Beckman pH meter could be utilized, the melted agar was placed in a water bath at approximately 42°C. With the addition of a small amount of acid, little trouble was experienced with the agar hardening prematurely.

Aerial mycelia was planted in a preliminary test of pH from 2.0 to 10.0. Further plantings for testing pH were made using the string technique of uniform plantings (12). The cultures were grown on agar slants in tubes. When covered with mycelia, short strands of cotton string (2-3 cm. in length) which were previously autoclaved in a malt solution were placed on the surface of the culture slants. After approximately two weeks, mycelia uniformly covered the strings.

At the time of planting, forceps sterilized by dipping in alcohol and flaming were used to extract the strings from the culture tubes. With a sterile scissors, short pieces of the string approximately two millimeters in length were transferred to plates divided in quarters. Duplicate plates were planted for each treatment.

Accession number 33 (Manistee National Forest) and Accession number 46 (Neal Pinetum, East Lansing) were used in this study.

In a series extending from pH 2.0 to pH 10.0, Polyporus schweinitzii was found to grow in a limited pH range. Growth in Accessions 33 and 46 was similar. Figure 1 illustrates the resultant growth in Accession 33 at pH 3.0, 4.0 and 5.0.

The data in Figures 2 and 3 and Table 2 were the results of seven days growth at 26°C. The plates were exposed to indirect light for about one-half minute duration for the purpose of examination at two different periods.

An analyses of the combined data of Accessions 33 and 46 are also shown in Table 2. Growth of mycelia between replicates showed significant difference. Therefore the Between Replicates Variance was used to arrive at a valid "F value" for Between pH, Between Cultures, and Interaction between Cultures and pH. There is a highly significant difference in the amount of growth at various pH levels and between the cultures

used. A significant difference was found between growth at pH 3.0 and pH 4.0.

Although there was not a significant statistical difference in growth between pH 4.0 and pH 5.0, the sharp reduction in growth above pH 5.0 promoted further study to determine the optimum pH with more exactness.

A series of plates varying in pH from 3.2 to 4.6 using more refined techniques than in the preliminary tests were inaugurated. The results are shown in Figures 4 and 5.

In view of the fact that a difference in growth habits of accessions has been shown in previous work, the data from the pH 3.2 to pH 4.6 series were analyzed separately. The data and analysis for Accession 36 is presented in Table 3.

Under the conditions of this experiment, there was no significant difference between growth from 3.6 to 4.0.

In an effort to obtain the lower extent of the optimum range of pH for growth a series of plates were set up at 0.1 pH unit intervals. However, it was difficult to attain this refinement and it can be seen from the data in Table 4 that results indicate only the general trend.

These data seem to indicate a definite downward trend in growth below pH 3.5. It may be concluded that pH 3.7 to pH 4.0 is optimum for growth of this culture.

Growth of Accession 46 was analyzed in a manner similar to the analysis of the data for Accession 36 and is presented in Table 5.

With the highly significant difference between replicates in mind, the"Between Replicates Variance" term was used in calculating the F value for between pH.

Cartwright and Findlay (7) make the statement that pH 4.0 is more favorable for growth of Polyporus schweinitzii than pH 7.0. The data from tests in these studies substantiate this information. Childs (3), using a buffered liquid synthetic media and eight different cultures, reported that the optimum pH for growth was greater than 4.0 and less than 5.4. Upon close examination of his tables, it was found that the optimum for some of his cultures agree with the results obtained in the present study.

Soil samples were gathered at the base of invaded trees and an equal number of apparently healthy trees. No difference in pH between the two groups was noted. The pH near the base of infected trees in the Teal Pinetum ranged from 4.3 to 6.3.

VIII Temperature

Tests were conducted to determine minimum, maximum, and optimum temperatures for growth of Polyporus schweinitzii in culture. Culture 36 was used in the series. The

methods employed were similar to those described in the pH studies. Constant temperature controls with variation approaching 0.5°C. provided means for studying this environmental factor.

The results obtained from pH studies showed no significant difference due to interaction of culture and pH. Therefore a single culture with duplicate plates and four plantings per plate was used in this study.

Growth readings across the diameter of the culture were made at four days and again at six days. The four day readings followed establishment of vigorous growth in the most aggressive cultures. A six day reading was chosen to enable accurate readings to be taken before growth of the individual plantings on the plates over ran each other. Plates showing no growth at six days were left in temperature controls for a period of ten days following the last reading.

Initial tests were made at the following Centigrade temperatures; 10,14,22,24, 26,28,30,32,34. These temperatures were used because equipment was available at these increments.

Results are shown graphically in Figures 6 and 7. Analysis of the data collected at the end of four days is presented in Table 6.

It can be seen from this data that there is a significant difference in the amount of growth between temperatures of 24°C., 26°., and 28°C. The optimum

growth is at 28°C.

An analysis of the data collected at six days yields fundamentally the same information and is presented in Table 7. Significant difference between growth at 24°C., 28°C., and 32°C. again appears. Optimum growth was at 28°C.

Growth was observed in the plates subjected to 30°C. temperature after a period of six days. Although the 32°C. and 34°C. plates were checked for a period of sixteen days, no growth was noted. At the end of the sixteen day period, the agar was hard and dry.

The plates at 10°C. were completely covered with growth at sixteen days. Through the courtesy of the Horticulture Department, Michigan State College, duplicate plates were placed in cold storage rooms with temperatures of 4.4°C. and 0.55°C. No growth was noted at sixteen days. At the end of two months, growth was established on the plates at 4.4°C. The mean diameter of the growth in centimeters was 0.63. The plates at 0.55°C. had no growth at the end of the two month period.

For the most part, the results show agreement with previous work on temperature relations of Polyporus schweinitzii. Cartwright and Findlay, using a method similar to that used in this study (3), found the optimum temperature to be 28°C.(7). It will be noted that their tests were conducted at 24°C., 28°C., and 32°C. in the neighborhood of the optimum. Humphrey and Diehers (25), found an optimum of 24°C. and Liese (29)

determined 29°C. to be the optimum.

A minimum temperature of 1°C. compares with Cartwright and Findley (7). They do not mention the period of time covered in their experiments but list this figure as the minimum using average daily increments for analysis. Their maximum temperature for growth was 32°C. However, Liese (24) found a maximum inhibiting temperature of 34°C.

Although growth was observed in the vicinity of 5°C., growth was too slow to be compared with growth at any higher temperatures.

IX Spread and Entrance of the Disease

Polyporus schweinitzii was discovered in the Beal Pinetum in 1928 by Baxter(1). Strong (45) reports the progress of the disease as evidenced by decay revealed following the severe windstorm of November 11, 1940. He also includes the location of a single tree found to be infected in 1939.

A survey was made following the silvicultural thinning on the fiftieth anniversary of the stand. The results imposed upon the evidence noted in late 1940 serve as an indication of the rate of spread in this stand.

It will be noted from Figure 3 that the spread of the disease in the past six years has been small.

Another heavy windstorm would blow down severely rotted trees and thus aid in a more exact determination of the infected trees than is possible at this time.

The tree designated with the numeral "9" in Figure 7 and 8 was removed from the remainder of the decayed portion of the stand showed no signs of fire scar or injury around or near the butt. It was not in a position in the tract for the soil to receive much trampling by people or animals.

An effort was made to determine if the depth of the roots or the direction of spread from the tree would give any indication as to the entrance of organism. The root system was uncovered by digging to an approximate depth of forty one inches and washing the roots free of soil with a Hardie sprayer using 100# pressure and a shade tree spray gun. Figure 9 illustrates the procedure followed as well as indicating the appearance of decay.

Infection was found to be present in all directions. Branch roots cut from the lower butt on the northeast, east, south, southeast, southwest, and northwest sides were infected. Roots near the surface were healthy for the most part. Lower roots were all infected. A typical infection pattern is illustrated in Figure 10. A comparison of infected and healthy roots is shown in Figure 11.

It should be emphasized in the discussion of the depth to which infection on roots extends, the obser-

vations were made of only one tree and might well be influenced by peculiarities of the particular site. The soil profile revealed a thin layer of clay hardpan at a depth of about one foot. The water table was located at forty-one inches.

Root pockets (Figure 18) were found in relative abundance near the attachment of the roots to the butt. Previous workers have placed considerable importance on possibility of spread of Polyporus schweinitzii from tree to tree through pockets of this type if one of the roots forming the pocket is infected (5). Advanced and incipient decay were observed in the vicinity of these root pockets in many cases.

X Inhibitory Effect of Various Chemicals and the Development of an Isolation Technique

Initial attempts at isolation showed evidence of invasion by secondary organisms, both fungi and bacteria, into the regions of advanced and incipient decay. The growth of the secondary invaders in culture plates was more rapid than the growth of Polyporus schweinitzii.

A search for an inhibitor of the common secondary infecting organisms that showed little inhibiting effect on Polyporus schweinitzii was instituted.

Coons and Strong (18) found malachite green, crystal violet, brilliant green, acriflavine, and copper sulfate to act as differentiating agents in Fusaria

species. DeWitt and Sherman (14) conclude that specificity of copper salts in bactericidal and fungicidal action is apparent. Keeney (27) has found sodium propionate to be fungistatic to a number of common pathogens. Calcium propionate has also proved to be effective in the same manner. Heibert and Poussey(26) report that di phenyl ($C_6H_5 \cdot C_6H_5$) vapor has fungistatic action on citrus fruit pathogens in pure culture and also has a wide range of tolerance to different fungi. It not only checks the vegetative growth but evidently prevents normal spore formation in the species of fungi tested.

With the results and conclusions of previous workers in mind, the following group of compounds were tested as to the maximum concentration at which they were not inhibitory to Poly orus schweinitzii, the effect on its future growth and development, and the inhibitory effect on common natural contaminants:

Crystal violet
Malachite green
Acriflavine
Brilliant green
Calcium propionate
Sodium propionate
Copper sulfate
Copper chloride
Diphenyl.

Crystal violet and acriflavine were obtained from National Aniline Division, Allied Chemical and Dye Corporation. Grubler's brilliant green, Coleman and Bell malachite green, and DuPont's commercial calcium and sodium propionates were used. Chemically pure copper

sulfate and copper chloride and Eastman Kodak Company dimethyl crystals completed the series.

Tests were made with four plantings per plate and duplicate plates of each treatment. Two accessions of Polyporus schweinitzii, namely 35 and 46, were used in each test to check the consistency of results between cultures.

A method of uniform measuring technique was devised to reduce experimental error in taking readings. Each plate was divided into four segments such that each of the four plantings per plate had an equal amount of space for growth. Measurements were made along a line which was the chord of the arc formed by each segment.

Subsequent tests were made with the above technique to insure equal amounts of mycelium for each planting as previously described in the experiments with various degrees of pH.

The cultures were exposed to the air for a short period to secure evidence as to the inhibiting effect of the chemicals to common contaminating organisms from the air.

Stock solution of the aniline dyes were made up in 0.5% concentration. Sterile water was used for the dilution. The concentration of the stock solutions was too great to permit growth of contaminants in thinly stoppered flasks. The stock solutions were diluted in such a manner that a constant ratio of sixteen cubic

centimeters of solution to sixty four cubic centimeters of malt agar was maintained. Dilutions of 1:1,000; 1:10,000; 1:50,000; 1:100,000; 1:500,000; 1:1,000,000 composed a series.

Calcium propionate and sodium propionate stock solutions were made in the same manner as those of the aniline dyes. Copper chloride and copper sulfate stock solutions were one-tenth Molar solutions. A constant ratio of sixteen cubic centimeters of solution to sixty four cubic centimeters of agar was also maintained in these series. However, the results of the aniline dye tests prompted the use of the following dilutions: 1:100; 1:1,000; 1:10,000; 1:50,000; and 1:100,000.

Sixty milligrams of diphenyl crystals were placed in the center of malt agar plates planted with the strips technique. The plates were not inverted in order to keep the crystals in the center of the plates.

Results of growth on crystal violet for periods of seven and fourteen days are shown graphically in Figures 13, 14, 15, and 16. Growth within each plate was uniform. The tests showed good inhibition of contaminators except in the 1:100,000 dilution. In this dilution what was considered to be a single species of mold was prevalent. The initial test plates were held for a month. At the end of that period, results were essentially the same as for the fourteen day period. However, growth at 1:50,000 concentration in both accessions 33 and 45

was more luxuriant and vigorous.

A series of tubes from 1:1,000 concentration to 1:20,000 in 1,000 increments determined more closely the maximum tolerance (Tubes 1 and 2). Malachite green proved to be more inhibitory to Polycarus schweinitzii than to organisms likely to be secondary invaders. Results are illustrated in Figures 17, 18, 19, and 20.

Brilliant green supported growth of Polycarus schweinitzii at relatively low concentrations(Figures 21, 22, 23, and 24). Contamination was evident at 1:100,000 and more dilute concentration. Subsequent tests showed a difference in growth of cultures 37 and 46. Culture 46 had a mean growth of 0.3 cm. in concentrations of 1:30,000. Culture 37 would not support growth in concentrations greater than 1:50,000.

Preliminary tests with acriflavine eliminated it as an aid to isolation. Growth of contaminating fungi was profuse at 1:1,000 concentration. Growth of Polycarus schweinitzii did not appear until dilutions of 1:100,000 were reached and was not profuse in any concentration.

Calcium propionate and sodium propionate showed little fungistatic activity. It was noted that they did exhibit bacteriostatic activity. Calcium propionate at 1:1,000 supported growth of both cultures. Sodium propionate supported growth at 1:10,000.

Copper sulfate was inhibitory to Polycarus

schweinitzii at 1:100 concentration but not at 1:1,000 (Figures 24, 26, 27, and 28). These results were refined by testing growth between 1:100 and 1:1,000 concentrations at various dilutions. A concentration of 1:500 proved to be the highest concentration capable of supporting growth of Polyporus schweinitzii. At this level the growth was limited and not uniform.

Copper sulfate was inhibitory to contaminating fungi and bacteria at 1:100 concentrations. However, upon leaving the treated plates exposed to the air for a period of thirty minutes, contamination was evident.

Copper chloride (Figures 29, 30, 31, 32) produced essentially the same results as copper sulfate. Further tests were not conducted because copper sulfate showed more promising results.

The data in Table 10 were obtained from plates in which diphenyl crystals were introduced into the center. Growth in fourteen days showed the same trends the measurements made at seven days. An analysis of the data in Table 10 was made and included in the table.

Although there is a highly significant statistical difference in growth between treatments, there were no contaminating colonies in the diphenyl treated plates. A mean of four contaminating colonies per plate was noted in the checks. The analysis showed no significant difference between Accession number 35 and number 46. Therefore a single accession was used in further tests.

Various concentrations of diphenyl crystals were introduced into the plates which were left exposed to the air for a period of thirty minutes. Growth of Polyporus schweinitzii decreased as the amount of diphenyl crystals increased (Table II).

The check plates were highly contaminated. The plates with diphenyl crystals were free from contamination or, if contaminated, the colonies were small and did not produce abundant spores.

An attempt to incorporate the bacteriostatic action of crystal violet and the fungistatic action of diphenyl by employing effective amounts of both crystal violet dye (1:500,000) in the medium and diphenyl crystals (30 mgs.) on the surface of the medium proved of no value. The plates were left exposed to the air for sixty minutes to insure heavy contamination. Growth of Polyporus schweinitzii was inhibited as well as that of contaminating bacteria and fungi.

In view of the results obtained with the use of diphenyl crystals, its value in isolations was tested. Twenty plates from each of three sources of infected white pine wood tissues were planted with four plantings per plate.

Accession Number	Source
78	Stump of same tree as Culture 46.
80	Tree on south side of Real Pinetum.
84	Roots of same tree as Culture 46.

Diphenyl crystals were added to ten of the plates of each accession, the remaining ten plates contained no

diphenyl crystals.

White pine tissue plantings were made from regions of decay following surface sterilization by burning alcohol. Approximately fifteen milligrams of the crystals were placed in each test plate. Plates with and without diphenyl crystals were planted alternately to minimize experimental error. Check plates were inverted; test plates were left upright. Results are tabulated in Table 12.

It can be seen from Table 12 that addition of diphenyl crystals aided in the isolation of Polyporus schweinitzii in all cases. The isolates obtained with the use of diphenyl were easier to separate from any contamination in the plate.

A period of approximately ten days is necessary for Polyporus schweinitzii to develop the characteristic yellow color sufficiently to be recognizable. The coloration of cultures has been described as barium yellow with thickened areas honey yellow to buckthorn brown to vivid when exposed to light (18). Childs uses over twenty color designations in a study of variability of isolates (9).

The plates with diphenyl crystals added seem to retain more delicate shades than those without diphenyl crystals. However, these shades are not pronounced enough to cause difficulty in identification.

XI Growth in Soils

Previous work in which the infection of seedlings and two year old plants was obtained through the roots(46) and the postulations of Boyce (6) lead one to believe that Polyporus schweinitzii is capable of growth in the soil. Consequently, a series of experiments was instigated to test the growth of the organism in soil.

Soil for pot plantings was obtained from the Deal Pinetum. Ten eight-inch pots were filled with soil from the A horizon[1], ten pots were filled with a mixture of the A and B horizons, and ten pots were filled with sand steam-sterilized for one hour.

White pine seeds obtained from the Boque Forest Nursery, Michigan State College were treated with a 1:1000 solution of mercuric chloride for three minutes and washed three times with sterile water. Seeds were planted thirty to a pot and covered with a shallow layer of sterile sand.

The pots were left in the greenhouse for a period of five months. Germination and emergence of seedlings was good. However, damping off of many of the plants occurred during the course of the experiment. Tissue plantings were made from dying plants. Fusarium species were isolated in a considerable number of cases. Fruiting bodies of fungi of the Order Nidulariales were found

[1]Horizons as defined in "Soils and Men", USDA Yrbk., 1938.

growing on the soil and on pot labels in a number of instances. Polyporus schweinitzii was not isolated in any case from the tissue planting made from these dying plants.

Attention was then directed to the possibility of growth of the organism in soil under controlled laboratory conditions. A number of factors were tested in an effort to obtain growth of Polyporus schweinitzii in vitro. Different layers of the soil profile were checked. Various techniques of handling the soil media were investigated in an attempt to find one that would support growth. Soil obtained from different plantations were checked.

In view of successful growth in a needle-duff agar media (46), samples composed for the most part of the A_{00}, A_0 and A_1 horizons were collected from the white pine stand in the Sandhill Plantation, Michigan State College. No evidence of the presence of Polyporus schweinitzii has ever been found in this plantation. These soil samples were collected on December 12, 1946 following an inch rainfall during the preceding twenty four hours. The soil samples collected from the A_{00} horizon had a moisture content such that no moisture addition was deemed necessary. The A_0 and A_1 horizon soil samples were watered with sterile water. The water was allowed to percolate for a period of five hours before use.

These soil samples were then placed in sterilized covered capsule jars. Twelve replicates of each horizon were prepared. Six of the replicates were sterilized in the autoclave for thirty minutes at fifteen pound pressure; six were not sterilized. Aerial mycelium from plantings on oat meal medium grown in one thousand cubic centimeter Erlenmeyer flasks provided the inoculum which was from Accession number 38. The mycelium was planted at the edge of the dish and its location marked with wax pencil. The capsule jars were then placed in 26°C. temperature incubations. These plantings were made on December 17, 1946. Addition of sterile water was found necessary at the end of the first month to maintain a suitable moist condition in the capsule jars.

At the end of three weeks, growth was noted in only one jar. A single replicate of sterilized A_1 horizon soil showed growth of one to two millimeters from the original planting. Jars were checked at ten day intervals. A final inspection was made at the end of seventy five days. Any macroscopic growth of fungi on the soil was replanted on malt agar plates. With the exception of the case described, no growth of Polyporus schweinitzii was found from these attempts to reisolate it.

Hiley's success with a test tube technique for the growth of Fomes annosus in soil (24) suggested possibilities of a somewhat similar test with Polyporus schweinitzii. A small plug of cotton was placed at the bottom of each

tube. Dry soil samples were poured in to a depth of two inches. Sterilized water was poured over the soil and allowed to percolate through the soil until the cotton plug was soaked.

With the previous results in mind, twelve tubes were set up containing A_1 horizon soil. Six of the tubes were sterilized as in the previous experiment; six tubes were not sterilized and remained as natural soil. Plantings of aerial mycelium were made on the surfaces of the soil in these tubes.

Results were observed at frequent intervals. Final observations were made sixty days following the initiation of this phase of the experiments. Although no case of growth sufficient for photomicrographing was evident, growth of white mycelium was observed in four of the sterilized tubes. Plantings were made from these tubes which showed mycelial growth in order to ascertain whether the growth was that of Polyporus schweinitzii or of a contaminant. Polyporus schweinitzii was reisolated from three of these tubes.

In an attempt to offset adverse environmental factors such as lack of aeration and improper moisture relations, a series of plantings was made in Petri plates. A thin layer of absorbent cotton was placed in the bottom of these plates to preserve moisture. A somewhat thicker layer of soil was poured over the cotton layer. Distilled water was sprinkled on the soil

to moisten it and to soak up the cotton for a reserve supply. String plantings as described in the pH experiments were again used here. Duplicate plates of each treatment were used in all cases.

Additional factors were introduced into this series. A sterilized and unsterilized set of each soil was again used. With A_0 horizon soil similar to that used in the original tests, duplicate plates with agar devoid of the usual nutrients added to the surface were tried to check the possibility of a detrimental moisture relationship in other tests. Previous work had shown good growth in various agar media which gives grounds, by virtue of the growth of the fungus, for an assumption that proper moisture conditions do exist with agar substratum.

Besides the experiment just described, a pair of duplicate plates with fifteen milligrams of diphenyl crystals placed on the surface of the soil was also initiated.

Polyporus schweinitzii showed slight growth on the A_0 horizon sterilized soil. No growth was noted in any other tests in this series.

The lack of measureable growth in Petri plates and the difficulty of keeping them moist and free of contamination over long periods of time prompted a return to the use of test tubes for the remaining experiments.

Soil from another white pine stand, Beal Pinetum, Michigan State College, was used in order to determine

growth in another soil in which white pine trees were growing. No growth of the fungus Polyporus schweinitzii was observed in the Red Pinetum soil. In the Sandhill Plantation soil a very slight growth of the organism in the sterilized A_0 horizon was observed. Chlamydospores were found on Polyporus schweinitzii mycelium in one of the Petri plates and reisolates were obtained.

Further experiments were conducted in an effort to check the growth of Polyporus schweinitzii in soils with different pH levels and to test for variation in ability to grow on soil between various accessions. A series was also inaugurated to ascertain whether Accession 45 might have lost its viability in culture on malt agar.

Sterilized and unsterilized samples of soil of the A_0 horizon varying in pH were tested for growth of Polyporus schweinitzii in them. The soil pH is the pH of the soil samples collected as they occurred naturally. Soils were selected that covered the soil pH range insofar as was possible. Table 13 shows the results obtained.

Growth of hyphae at pH 4.3 in the sterilized series was noted to be about three millimeters from the locus of the planted fungus. Similar growth was noted in the sterilized series at pH 5.3 and pH 5.9. At pH 5.9, the growth of the fungus on some of the needles was macroscopically evident. However, growth was sparse in all instances and not of sufficient quantity to be able

to compare with growth on malt agar medium.

All the previous work with growth in soil in this series of experiments has been done with Polyporus schweinitzii Accession number 36. It was deemed desirable to check the growth of other accessions in the event that Accession number 36 might have different growth habits in soil. Results are presented in Table 14.

Although growth was noted in four of the five accessions tested, growth in no case was prolific enough to be photographed. Replicate 1 of the sterilized series of Accession 46 showed a thin mycelial mat across the top of the soil in the tube. Polyporus schweinitzii was reisolated from this culture and from several others to prove the growth was that of the organism tested and not of a contaminant.

A further possibility that the organism might have partially lost its viability in vitro occurred. A thin layer of malt agar was poured in the bottom of a Kolle' flask. Small blocks of sterilized white pine were then placed in the flask. This flask was planted with Accession number 46 and allowed to grow as one of the stock cultures.

Plantings from this stock culture were made on sterilized and unsterilized A_0 soil. A small amount of growth was noted on the sterilized series. None was noted on the unsterilized series.

Throughout these studies, the isolates of Polyporus

schweinitzii in unsterilized series remained altogether dormant and soon was a brown shriveled mass. Frequently they were attacked and overgrown by other fungi.

XII Summary and Conclusions

A list of the known hosts of Polyporus schweinitzii has been assembled. With the exception of a few genera and species, the organism attacks practically all conifers.

Although a number of synonyms are in use in the literature, Polyporus schweinitzii Fr. and Phaeolus sistotremoides (A. and S.) Murrill are the most common.

Nine out of twelve accessions produced insipient sporophore tissue in vitro. The sporophore tissue produced was abnormal in appearance.

A pH 3.5 to pH 4.0 of malt agar seemed optimum for growth. Growth was evident at pH 3.0 but not at pH 2.0. pH 6.0 was the maximum for mycelial growth.

The optimum temperature for mycelial growth was found to be 25°C. The maximum temperature was 30°C. Although growth was evident at approximately 5°C. following two months incubation, it was not of sufficient quantity to compare with growth at 14°C., the minimum for growth in six days. However, the fact that growth occurs at 5°C. in malt agar is of importance in relation to probable growth under climatic condition of the colder region in which this fungus lives.

Spread of red-brown butt rot in the stand of Eastern white pine is slow. The radius of evident spread of the diseased area in the past six years has been too small to be of commercial importance.

Secondary invaders made isolation of Polyporus schweinitzii difficult. A group of known fungal and bacterial inhibitors were tested to determine the maximum concentration at which they would support growth of Polyporus schweinitzii and inhibit growth of the secondary invaders. The maximum concentrations that would permit growth of Polyporus schweinitzii on malt agar medium are as follows; crystal violet, 1:50,000; malachite green, 1:500,000; brilliant green, 1:30,000 to 1:50,000; acriflavine, 1:100,000; calcium propionate, 1:1,000; sodium propionate 1:10,000; copper sulfate, 1:500; and copper chloride, 1:1,000. Diphenyl crystals were tested in amounts of 120 milligrams per plate and found to permit growth of Polyporus schweinitzii at that concentration.

An evaluation of the chemicals used as aids to isolation was made from the data concerning concentrations permitting growth of Polyporus schweinitzii and observations of the growth of secondary invaders. Malachite green, acriflavine, calcium propionate, sodium propionate, and copper chloride proved of little aid in isolations. Crystal violet, brilliant green, and copper sulfate showed promise as aids to isolation. Diphenyl

crystals proved more promising than any of the other inhibitors. Fifteen milligrams of diphenyl crystals placed in the center of a poured Petri plate will substantially increase the number of pure culture isolations from tissue plantings.

A series of experiments was instigated to test the growth of Polyporus schweinitzii in soil. The organism could not be isolated from a series of seedlings grown on soil from an area on which many trees had red-brown butt rot caused by this fungus. Sterilized and unsterilized series were inaugurated for a group of experiments testing the growth of the organism in the soil. Various soil horizons, techniques of culturing organisms, white pine plantation sites, pH's, accessions, and wood cultured isolates were tested for growth. Growth of Polyporus schweinitzii was not noted in any test of unsterilized soil. Light growth--too small to be photographed--was noted in A_0 and A_1 horizons of the white pine plantation sites checked. At lower pH concentrations all except one accession and a wood cultured mycelium showed slight growth. In no case was the fluffy, thick, vigorous growth expressed by the organism on malt agar even suggested.

Soil was a poor medium for growth in vitro.

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Tables

Table 1. List of host plants susceptible to attack by Polyporus schweinitzii Fr.

Species	Common Name[1]	Authority
Abies alba Mill.	Silver Fir	35
Abies amabilis (Dougl.) Forbes.	Pacific silver fir	44
Abies arizonica Merriam	Corkbark fir	44
Abies balsamea (L.) Mill.	Balsam fir	44,3
Abies concolor Lindl. and Gord.	White fir	44
Abies grandis Lindl.	Lowland white fir	44
Abies lasiocarpa (Hook.) Nutt.	Alpine fir	44
Abies magnifica A. Murr.	Red fir	44, 21
Abies nobilis Lindl.	Noble fir	44,21
Abies shastensis Lemmon	Shasta fir	44
Chamaecyparis thyoides (L.) B.S.P.	Southern white cedar	28
Eucalyptus globulus Labill.		44,41
Larix decidua Mill. (L. europea D.C.)	European larch	44,42,35
Larix Laricina (DuRoi) Koch	Tamarack	44, 42
Larix Lyallii Parlot	Alpine larch	44,21
Larix occidentalis Nutt.	Western larch	44,49,47
Libocedrus decurrens Torr.	Incense cedar	44,49
Liquidamber styraciflua L.	Red gum	25
Picea abies L	Norway spruce	30
Picea Engelmanni (Parry) Engelm.	Engelmann spruce	44,49,47
Picea glauca (Moench) Voss (P. canadensis (L.) B.S.P.)	White spruce	21,49,3
Picea mariana (Mill.) B.S.P.	Black spruce	44,21,49
Picea parryana (Andre) Parry		44,21
Picea rubra (DuRoi) Dietr. (P. rubens (Sarg.)	Red spruce	44,21,49,31
Picea sitchensis Carr.	Sitka spruce	44,49,14
Pinus albicaulis Engelm.	Whitebark pine	44
Pinus aristata Engelm.	Foxtail pine	49
Pinus arizonica Engelm.	Arizona pine	44
Pinus attenuata Lemmon.	Knobcone pine	44,21
Pinus banksiana Lamb.	Jack pine	44,49
Pinus cembroides Zucc.	Mexican pinon	44
Pinus chihuahua Engelm.	Chihuahua pine	44, 21
Pinus contorta Dougl.	Lodgepole pine	44,49,47
Pinus contorta Loud. var Latifolia S. Wats. (P. murrayana Balf.)	Lodgepole pine mountain form	49
Pinus echinata Mill.	Shortleaf pine	44,49
Pinus edulis Engelm.	Nut pine	44,21
Pinus flexilis James	Limber pine	44

[1] Common names follow Harlow and Harrar(16) insofar as possible.

Table 1. continued

Species		
Pinus glauca Walt.	Spruce pine	44,21
Pinus Jeffreyi Murr.	Jeffrey pine	44,21
Pinus lambertiana Dougl.	Sugar pine	44
Pinus murrayana Sudworth. (P. contorta)		44,21,30
Pinus monophylla Torr. and Frem.	Singleleaf pinon	44,21
Pinus monticola Dougl.ex Lamb.	Western white pine	44,49,47
Pinus palustris Mill. (P. australis Michx.)	Longleaf pine	44,47
Pinus pinaster Ait.	Cluster pine	24,35
Pinus ponderosa Dougl.	Western yellow pine	44,49,47
Pinus resinosa Ait.	Red pine	44,49
Pinus rigida Mill.	Pitch pine	44,21,49,39
Pinus sabiniana Dougl.	Digger pine	44,21,49
Pinus strobiformis Engelm.	Mexican white pine	44
Pinus strobus L.	Eastern white pine	44,49,49,35
Pinus sylvestris Linn.	Scots pine	49,24 49,35,14
Pinus Taeda L.	Loblolly pine	44
Pinus virginiana Mill.	Virginia pine	44,49
Pseudotsuga taxifolia (Lam.) Britton	Douglas fir	44,21,49
Quercus alba L.	White oak	44,49,77
Taxus brevifolia Nutt.	Pacific yew	44
Thuja occidentalis L.	Northern white cedar	44,49
Thuja plicata D.Don	Western red cedar	44,21,49
Tsuga canadensis (L.) Carr.	Eastern hemlock	44
Tsuga heterophylla (Raf.) Sarg.	Western hemlock	44,49
Tsuga mertensiana (Bong.)	Mountain hemlock	44,49
Cedrus Loud.		49
Betula lutea Michx.	Yellow birch	13

Table 2. Analysis of growth of Accessions 73 and 46 at various degrees of pH in seven days.

	Accession 73			Accession 46		
	7.0	4.0	5.0	7.0	4.0	5.0
	2.1*	3.9	3.7	3.7	4.0	4.0
	2.0	4.0	3.6	3.5	3.1	3.7
	2.0	3.9	3.1	3.1	4.2	3.8
	2.4	3.6	3.4	3.5	4.0	3.5
Σx	8.7	15.3	14.0	13.7	15.3	15.0
Σx^2	19.21	71.85	51.50	51.15	62.59	57.34
	2.7	3.9	3.4	3.5	3.4	3.4
	2.5	3.7	3.9	3.5	3.4	3.3
	2.5	3.9	3.4	3.4	3.4	3.5
	2.7	3.9	3.4	3.3	3.4	3.9
Σx	10.4	15.4	14.1	13.7	14.6	14.7
Σx^2	27.52	59.78	50.27	46.51	53.44	54.03

Sums of Squares

Total = 484.09 - 471.62 = 12.47
Between plates = 481.94 - 471.62 = 10.32
Total (Culture x pH) = 480.81 - 471.62 = 9.19
Between Cultures = 481.21 - 471.62 = 9.59
Between pH = 477.62 - 471.62 = 6.00
Int. Culture x pH = 9.19 - 6.00 - 2.97 = 0.22
Between Replicates = 10.32 - 9.19 = 1.13
Within plates = 12.47 - 10.32 = 2.15

Source of Variation	Degrees of freedom	Sums of squares	Variance	F	5% Level	1% Level
Between cx	2	2.97	1.49	16.56	3.15	5.01
Between cultures	1	2.75	2.75	14.86	4.11	7.40
Int. Cultures X pH	2	0.22	0.11	0.70	3.26	5.25
Between Replicates	6	1.13	0.17	0.83	2.36	3.36
Error	36	8.15	0.063			
Total	47	12.47				

* Each value is the diameter of a lesion measured in centimeters.

Table 2 continued.

Minimal Significant Difference = 2.447 $\sqrt{\frac{0.17 \times 2}{12}}$

= [illegible]

pH	Mean
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]

Table 3. Analysis of data obtained from growth of Accession 31 in pH range from 3.2 to 4.6.

pH	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
	1.1*	[illegible]	3.1	[illegible]	[illegible]	2.[illegible]	1.[illegible]	
	0.8	3.2	2.6	2.3	2.[illegible]	2.[illegible]	1.[illegible]	
	1.0	2.[illegible]	3.[illegible]	2.[illegible]	2.4	2.[illegible]	0.[illegible]	
	0.9	[illegible]	[illegible]	1.[illegible]	2.[illegible]	2.[illegible]	0.6	
Σx	[illegible]	12.[illegible]	11.[illegible]	[illegible].2	[illegible]	[illegible]	[illegible]	
Σx^2	[illegible].27	[illegible].22	[illegible].61	[illegible].10	[illegible].47	[illegible].[illegible]	[illegible].[illegible]	
	2.[illegible]	[illegible].6	[illegible].2	2.[illegible]	1.[illegible]	2.2		
	0.6	[illegible]	[illegible]	[illegible]	2.7	1.[illegible]		
	1.[illegible]	[illegible]	[illegible]	[illegible]	2.[illegible]	2.9		
	1.[illegible]	2.[illegible]	2.1	2.4	2.[illegible]	1.[illegible]		
Σx	[illegible].7	17.1	12.2	11.[illegible]	[illegible]	[illegible].1		
Σx^2	12.[illegible]	[illegible].29	[illegible].[illegible]	[illegible].[illegible]	[illegible].21	10.15		

Sums of Squares

Total = [illegible].[illegible] - [illegible].[illegible] = [illegible].[illegible]

Between plates = [illegible].11 - [illegible].[illegible] = 22.41

Between pH = [illegible].[illegible] - [illegible].[illegible] = 27.19

Between Replicates = 22.41-27.19 = 2.22

Within plates = [illegible].[illegible] - 22.41 = 2.47

Source of Variation	Degrees of Freedom	Sums of squares	Variance	F	5% Level	1% Level
Between pH	5	27.19	5.43	20.[illegible]	2.47	3.[illegible]
Between Replicates	6	2.[illegible]2	0.37	1.42	2.[illegible]6	3.[illegible]6
Error	[illegible]6	2.47	0.26			
Total	47	[illegible].[illegible]				

* Each value is the diameter of a planting measured in centimeters.

Table 3 continued.

Minimal Significant Difference = $2.023\sqrt{\frac{0.26 \times 2}{8}}$

= 0.517

pH	Mean
3.2	1.27
3.6	3.24
3.8	3.01
4.0	2.59
4.2	2.32
4.5	1.36

Table 4. Growth at various degrees of pH. Accession 36.

pH	Growth							
	1	2	3	4	5	6	7	8
3.1	+++	+	+	+	+	+	+	-
3.2	+++	+++	++	++	++	++	-	-
3.3	+++	++	++	++	++	-	-	-
3.4	+++	+++	+++	++	++	++	++	++
3.5	++++	+++	+++	+++	+++	+++	+++	+++
3.6	+++	+++	+++	+++	++	+	+	+
3.7	+++	+++	+++	+++	+++	+++	+++	+++
3.8	+++	+++	+++	+++	+++	+++	+++	+++
3.9	+++	+++	+++	+++	+++	+++	++	+

Table 5. Analysis of data obtained from growth of Accession 46 in pH range from 7.0 to 4.6.

	Accession 46					
pH	7.0	7.5	7.0	4.0	4.5	4.6
	1.0*	6.0	6.0	6.0	6.0	1.5
	2.0	6.0	6.0	6.0	5.10	2.0
	2.8	6.0	6.0	6.0	4.1	2.0
	2.0	6.0	6.0	6.0	3.0	0.2
Σx	8.0	24.0	24.0	24.0	17.2	5.8
Σx^2	16.08	144.0	144.0	144.0	87.82	10.60
	2.5	3.1	6.0	6.0	4.0	3.2
	2.0	4.5	6.0	6.0	3.0	3.8
	0.0	4.0	6.0	6.0	2.5	2.5
	1.2	2.6	6.0	6.0	3.5	0.5
Σx	7.7	14.3	24.0	24.0	13.0	10.0
Σx^2	7.69	53.23	144.0	144.0	43.50	31.18

Sums of Squares

Total = 970.40 - 776.02 = 194.38

Between plates = 949.02 - 776.02 = 173.00

Between pH = 929.36 - 776.02 = 153.34

Between Rep. = 173.00 - 153.34 = 19.66

Within plates = 194.38 - 173.00 = 21.38

Source of Variation	Degree of Freedom	Sums of squares	Variance	F	5% Level	1% Level
Between pH	5	153.34	30.67	51.86	2.47	3.71
Between Replicates	5	19.66	3.93	6.66	2.76	4.14
Error	36	21.38	0.59			
Total	47	194.38				

$$\text{Minimal Significant Difference} = 2.447\sqrt{\frac{3.93 \times 2}{6}}$$

$$= 2.02$$

* Each value is the diameter of a plantlet measured in centimeters.

Table 5 continued

pH	Lesion
7.2	1.46
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
4.2	[illegible]
[illegible]	1.[illegible]

Table 6. Analysis of growth at Various temperatures in four days.

	Temperature (C.)			
	[illegible]°	24°	[illegible]°	[illegible]°
	1.[illegible]*	1.7	2.4	[illegible]
	1.[illegible]	2.1	1.7	2.1
	1.7	1.4	2.5	1.4
	1.[illegible]	1.9	2.[illegible]	1.[illegible]
Σx	[illegible]	7.1	[illegible]	7.[illegible]
Σx^2	[illegible]	12.[illegible]	13.74	12.[illegible]
	1.[illegible]	1.5	2.5	1.4
	1.6	1.[illegible]	2.[illegible]	1.[illegible]
	1.[illegible]	1.[illegible]	2.[illegible]	1.[illegible]
	1.[illegible]	1.4	2.[illegible]	1.5
Σx	[illegible]	[illegible]	[illegible]	6.[illegible]
Σx^2	7.[illegible]	7.59	[illegible].04	10.01

Sums of Squares

Total = 104.49 - [illegible].69 = 5.[illegible]
Between plates = 102.92 - [illegible].69 = [illegible].23
Between Temperatures = 102.31 - [illegible].69 = 3.62
Between Replicates = 4.23 - 3.62 = 0.61
Within plates = [illegible].80 - 4.23 = 1.57

* Each value is the diameter of a lesion measured in centimeters.

Table 6 continued

Source of Variation	Degrees of Freedom	Sums of Squares	Variance	F - Level	1 Level
Between Tem.	3	3.52	1.21	18.52	3.01	4.72
Between Reps.	4	0.41	0.13	2.71	2.78	4.22
Error	24	1.57	0.065			
Total	31	5.50				

Minimal Significant Difference = 2.064 $\sqrt{\frac{0.065 \times 2}{5}}$

= 0.262

Tem.	Mean
220.	1.46
240.	1.58
260.	2.72
280.	1.56

Table 7. Analysis of north t various temperatures at six days.

	Temperature (C.)					
	14°	22°	24°	26°	28°	30°
	0.2*	2.1	3.3	4.2	3.2	1.2
	0.3	3.2	3.7	4.5	3.4	1.5
	0.4	2.9	3.0	4.0	3.4	0.5
	0.3	3.1	3.4	4.2	4.0	0.3
Σx	1.2	12.3	13.4	16.7	14.7	4.0
Σx^2	0.36	37.67	45.14	69.59	54.33	5.26
	0.4	3.4	3.4	4.2	3.0	1.2
	0.3	3.2	3.2	4.2	3.1	0.3
	0.2	2.7	3.3	4.2	3.2	0.3
	0.4	3.5	3.3	4.2	3.1	0.4
Σx	1.1	13.5	13.2	16.8	12.4	3.1
Σx^2	0.41	45.55	43.54	70.56	38.46	3.73

* Each value is the diameter of a lesion measured in centimeters.

Table 7 continued

Sums of Squares

Total = 415.31 - 315.70 = 99.61
Between Blocks = 412.85 - 315.70 = 97.15
Between Temps. = 412.99 - 315.70 = 96.79
Between Reps. = 97.15 - 96.79 = 0.36
Within Blocks = 99.61 - 97.15 = 2.46

Source of Variation	Degrees of Freedom	Sums of Squares	Variance	F	5% Level	1% Level
Between Temp.	7	96.79	13.83	253.53	2.17	3.36
Between Reps.	5	0.36	0.14	2.06	2.36	3.36
Error	35	2.46	0.063			
Total	47	99.61				

Minimal Significant Difference = $2.025\sqrt{\frac{0.063 \times 2}{8}}$

= 0.263

Temp.	Mean
15°	0.29
22°	3.29
24°	3.79
26°	4.19
28°	3.13
30°	0.38

Table 8. Growth in various concentrations of crystal violet in fourteen days. Accession 33.

Conc.	Growth in Ml.							
1:10,000	-	-	-	-	-	-	-	-
1:20,000	-	-	-	-	-	-	-	-
1:30,000	-	-	-	-	-	-	-	-
1:40,000	-	-	-	-	-	-	-	0.5
1:50,000	0.4	tr	-	-	tr	tr	tr	-

Table 9. Growth in various concentrations of crystal violet in fourteen days. Accession 46.

Conc.								
1:10,000	–	–	–	–	–	–	–	–
1:20,000	–	–	–	–	–	–	–	–
1:30,000	–	–	–	–	–	–	–	–
1:40,000	–	–	–	–	–	–	–	–
1:50,000	0.6	–	–	–	0.8	0.7	–	–

Table 10. Analysis of growth of Helminthosporium sativum in presence of final crystals after seven days.

Accession 7					Growth				
Check		1.9	1.0	1.4	0.5	1.5	0.4	1.7	1.1
Dimethyl		1.0	0.5	0.7	1.0	0.2	0.2	0.0	0.5
	Σx	2.9	1.5	1.1	1.5	1.7	0.6	2.6	1.6
	Σx^2	4.50	1.25	0.73	1.25	0.29	0.20	3.70	1.37

Accession 46					Growth				
Check		2.2	0.5	0.6	1.2	0.4	1.3	0.7	0.5
Dimethyl		1.2	0.5	1.5	0.0	0.4	0.2	0	0
	Σx	3.4	1.0	2.3	1.2	1.2	1.5	0.7	0.5
	Σx^2	5.28	0.27	2.79	1.44	0.30	1.73	0.49	0.27

Sums of Squares

Total = 29.63 - 19.22 = 10.41
Between plates = 22.99 - 19.22 = 3.77
Total Treatment x Cultures = 22.22 - 19.22 = 3.00
Between Treatments = 21.44 - 19.22 = 2.22
Between Cultures = 19.37 - 19.22 = 0.15
Int. Treat. x Cultures = 3.00 - 0.15 - 2.22 = 0.63
Between Replicates = 3.77 - 3.00 = 0.37
Within Plates = 10.41 - 3.77 = 6.64

* Each value is the diameter of a planting measured in centimeters.

Table 10 continued

Source of Variation	Degrees of Freedom	Sums of squares	Variance	F	5% Level	1% Level
Between Treat.	1	0.22	0.22	[illegible]	[illegible]	7.32
Between Adults.	1	0.15	0.15	0.[illegible]	4.21	7.68
Int. Treat. x Adult.	1	0.43	0.43	1.59	4.21	7.68
Between Pens.	4	0.97	0.24	0.[illegible]	2.76	4.22
Error	84	8.64	0.27			
Total	91	10.41				

Table 11. Growth in various amounts of dinheyl crystals per petri plate.

Treatment	Growth in 24 Hrs.	Growth in 48 Hrs.
Check	1.[illegible]*	2.[illegible]
15 mg.	1.[illegible]	2.78
30 mg.	0.[illegible]	2.14
60 mg.	0.[illegible]	2.[illegible]
120 mg.	0.[illegible]	2.[illegible]

*Each figure is the mean of eight measurements.

Table 12. Number of isolates of *Salmonella* spp. recovered from tissue samples.

Plates	Accession Number 73 Without Diphenyl	73 With Diphenyl	80 Without Diphenyl	80 With Diphenyl	84 Without Diphenyl	84 With Diphenyl
1	3	–	–	–	–	–
2	1	4	–	–	–	–
3	–	3	–	2	–	2
4	–	–	–	3	–	–
5	–	4	–	–	–	–
6	–	–	–	2	–	–
7	–	–	–	–	–	–
8	–	4	–	–	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–
Total	4	15	0	7	0	2
Percent isolates	10.0	37.5	0.0	17.5	0.0	5.0

Table 13. Growth in various pH concentrations after fourteen days.

		pH 4.3	5.3	5.9	6.1	7.6
Replicate 1	Sterilized	–	–	+	–	–
	Unsterilized	–	–	–	–	–
Replicate 2	Sterilized	+	+	–	–	–
	Unsterilized	–	–	–	–	–

Table 14. Growth of various accessions in A_2 horizon after fourteen days.

		Accession Number				
		46	3	22	26	29
Replicate 1	Sterilized	+	—	+	+	—
	Unsterilized	—	—	—	—	—
Replicate 2	Sterilized	+	—	—	+	+
	Unsterilized	—	—	—	—	—

Figures

Figure 1.

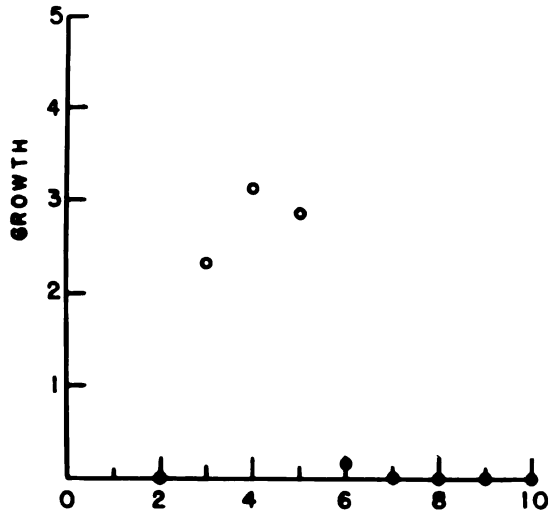

FIG. 2

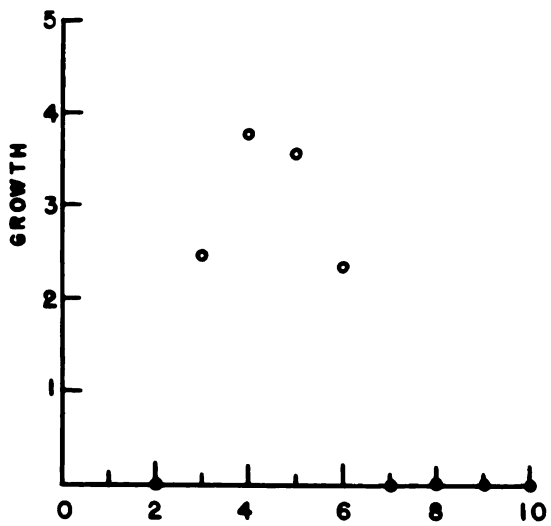

FIG. 3

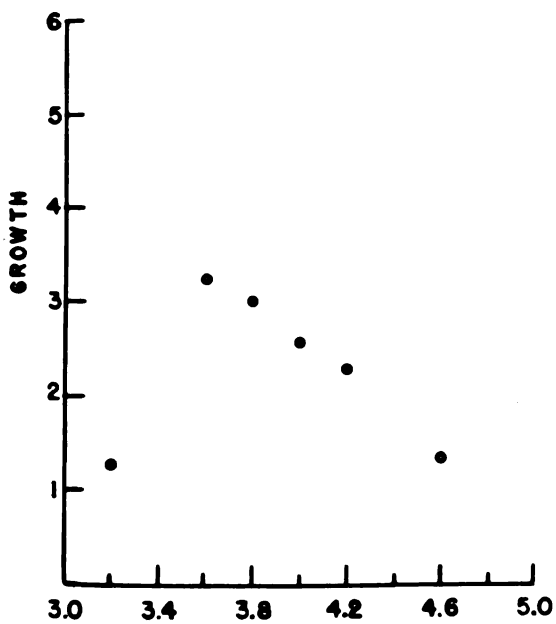

FIG. 4

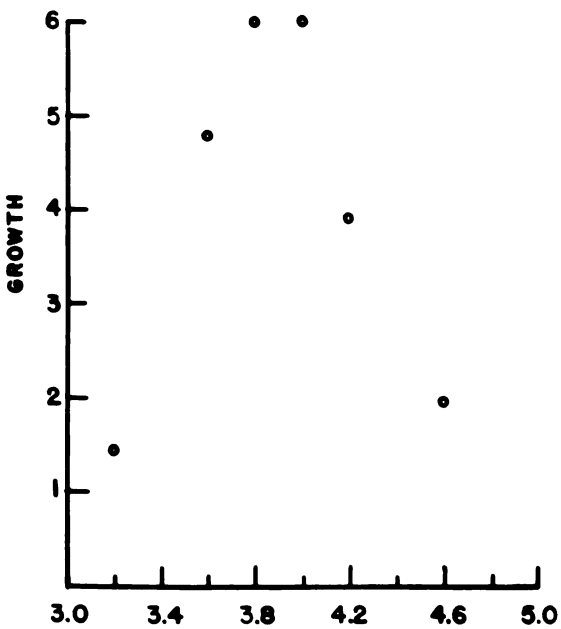

FIG. 5

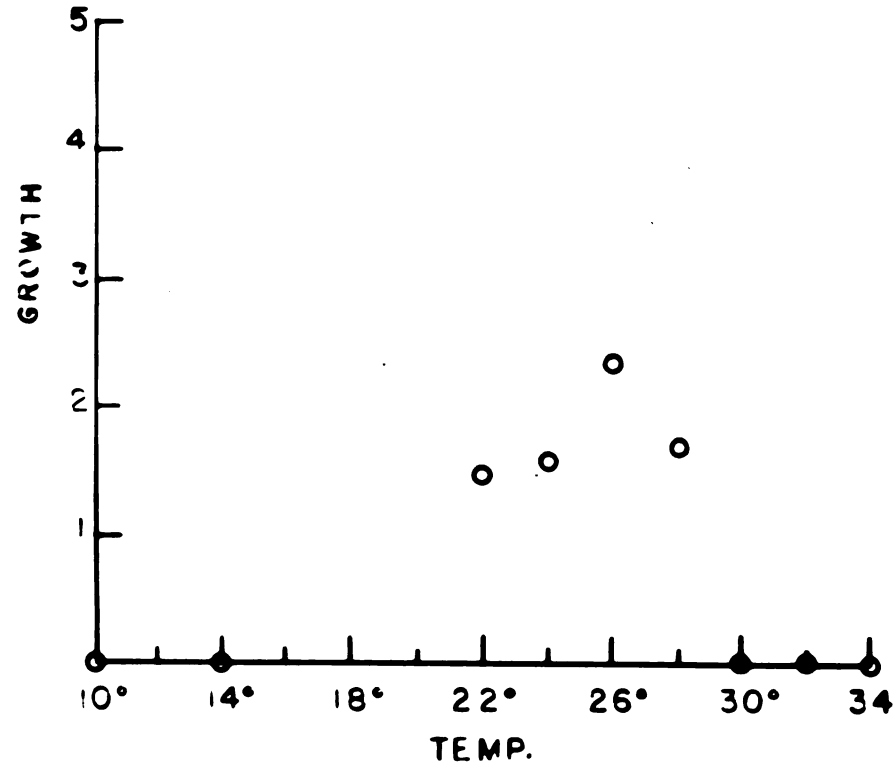
5
4
3
2
1
GROWTH
10°
14°
18°
22°
26°
30°
34
TEMP.

FIG. 6

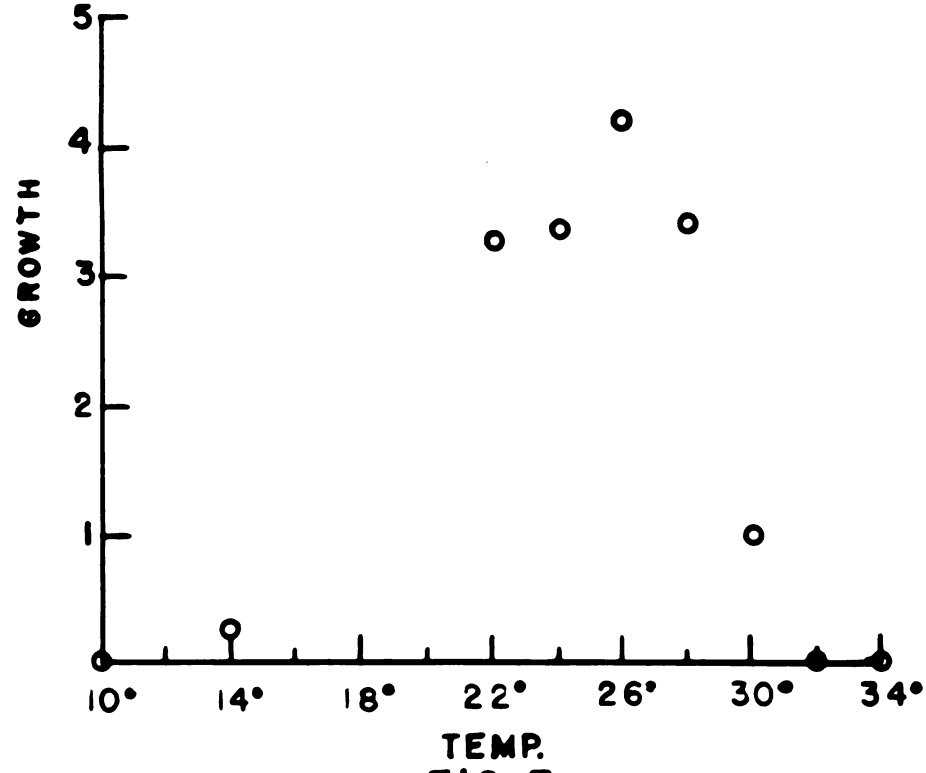
5
4
3
2
1
GROWTH
10°
14°
18°
22°
26°
30°
34°
TEMP.

FIG. 7

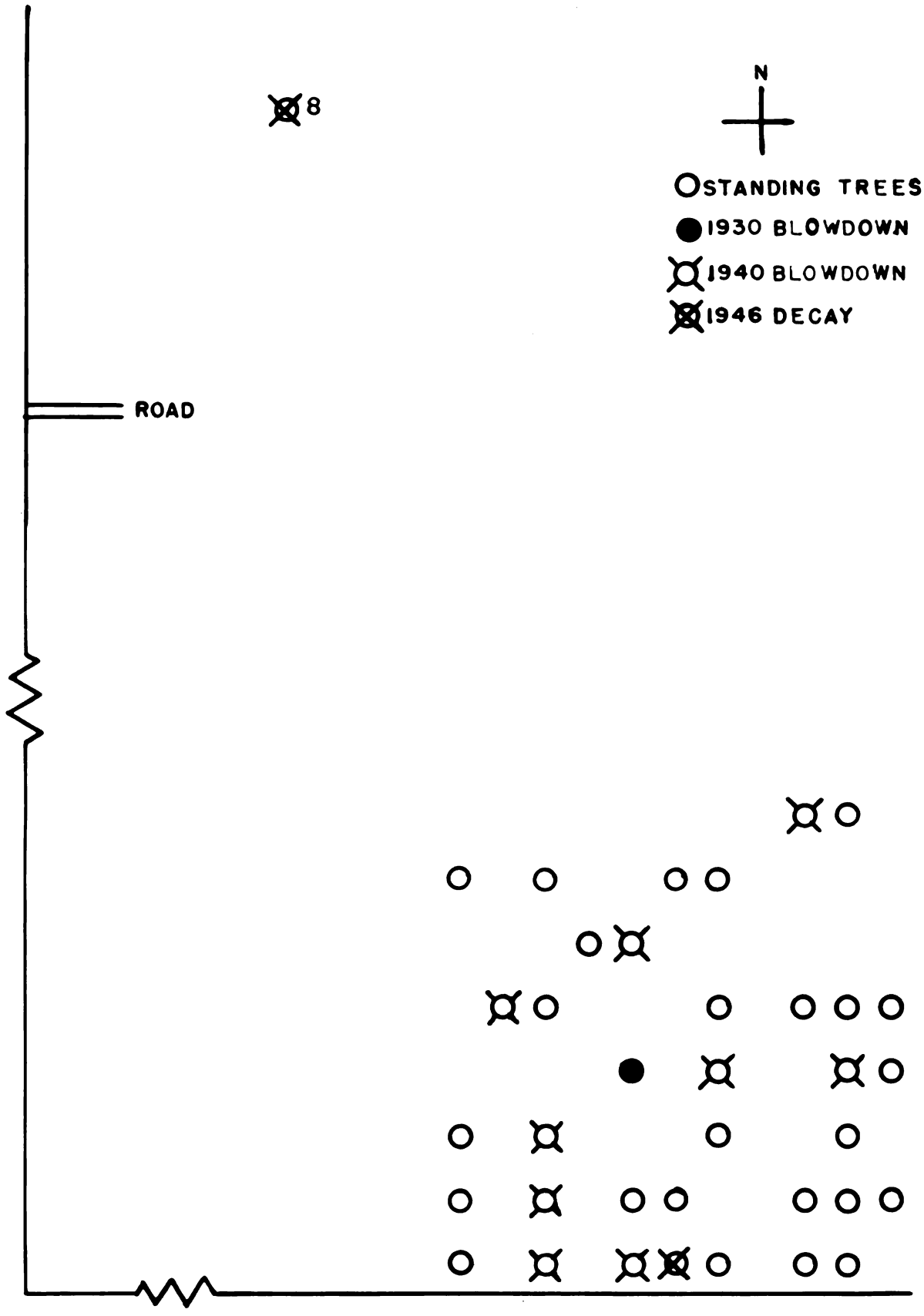
8
N
STANDING TREES
1930 BLOWDOWN
1940 BLOWDOWN
1946 DECAY
ROAD

FIG. 8

Figure 9.

Figure 10.

Figure 11.

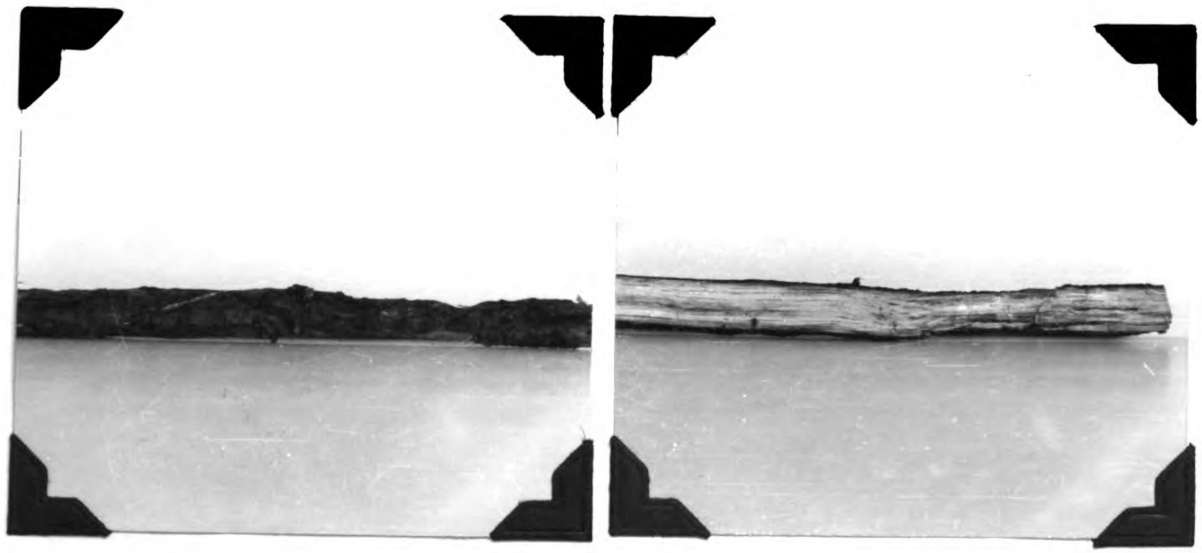

Figure 12.

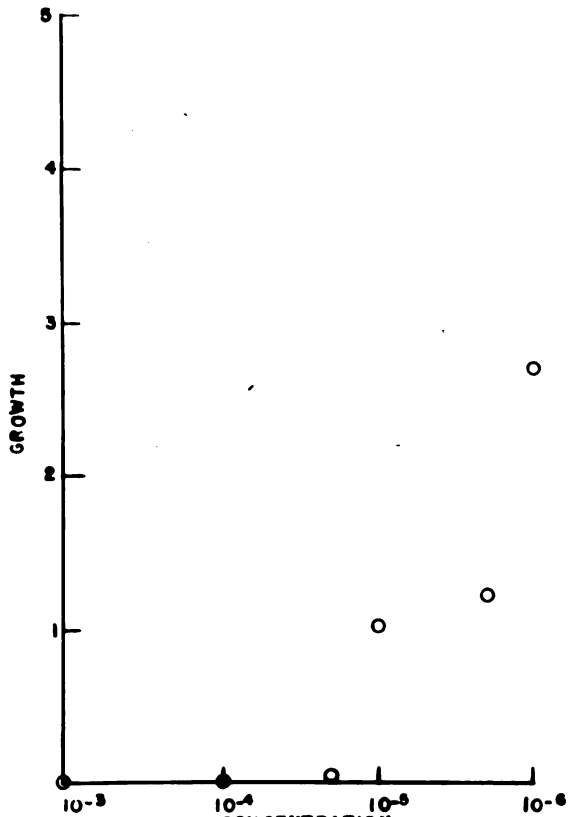

FIG.13

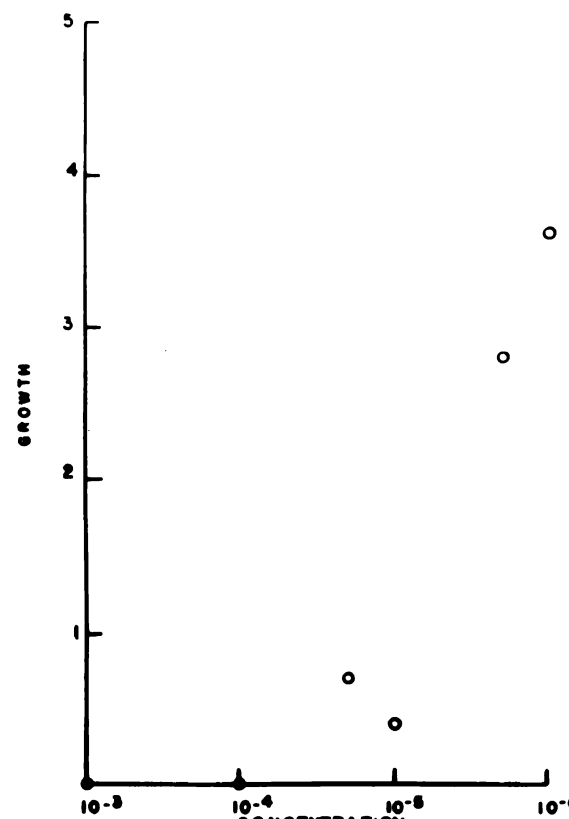

FIG.14

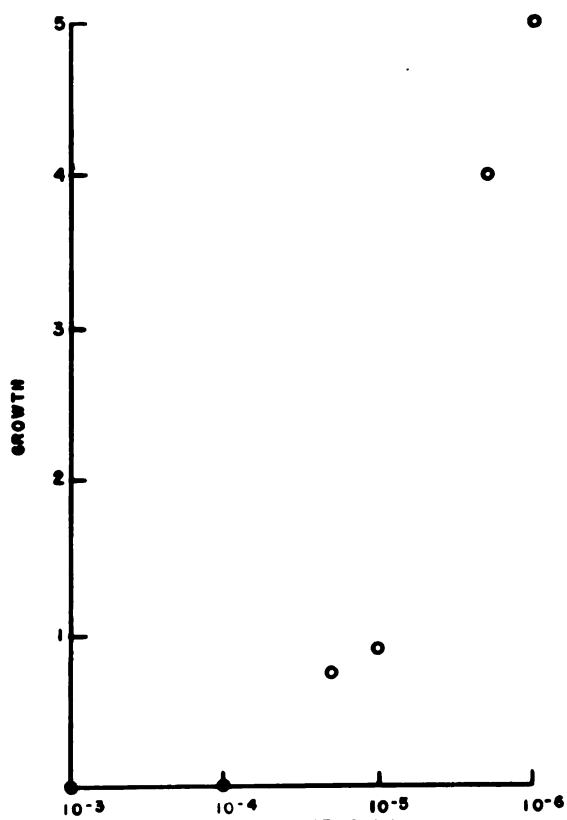

FIG.15

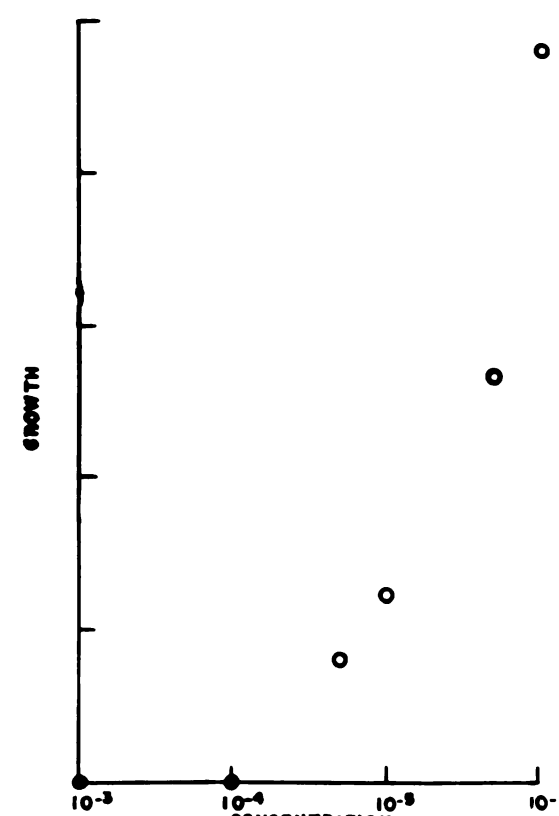

FIG.16

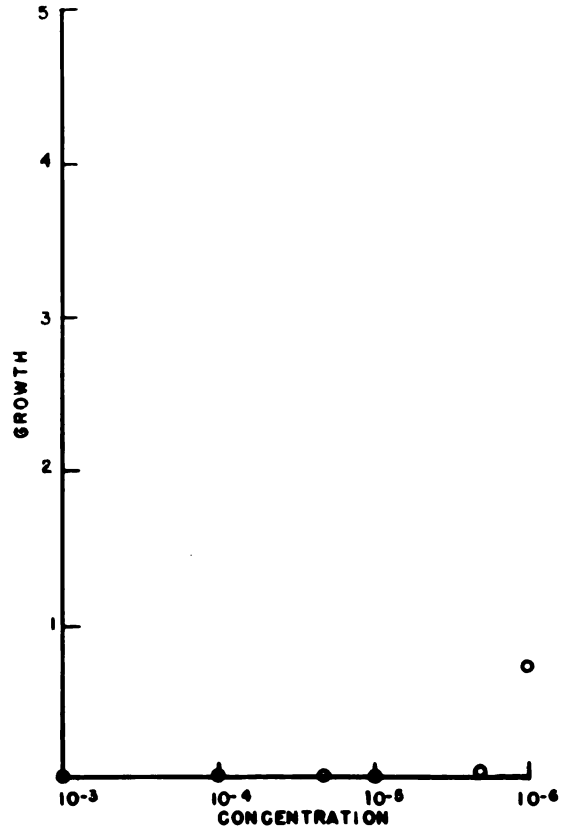

FIG. 17

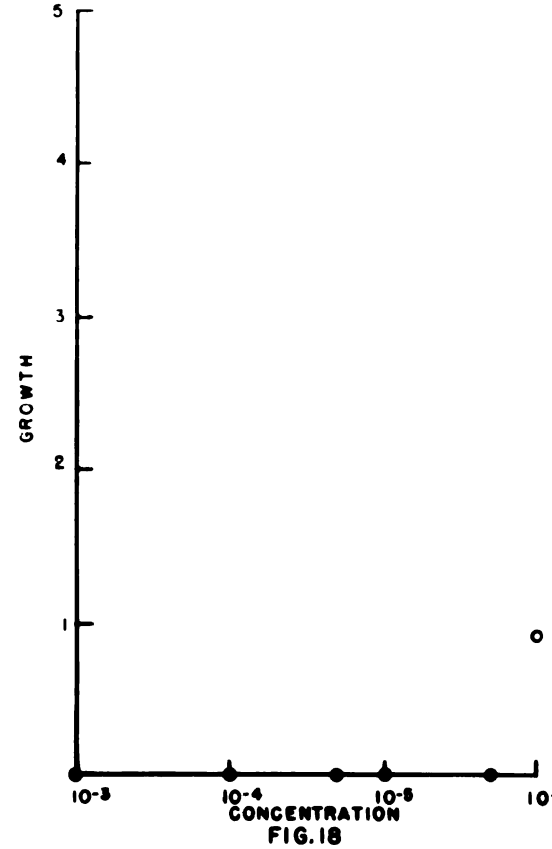

FIG. 18

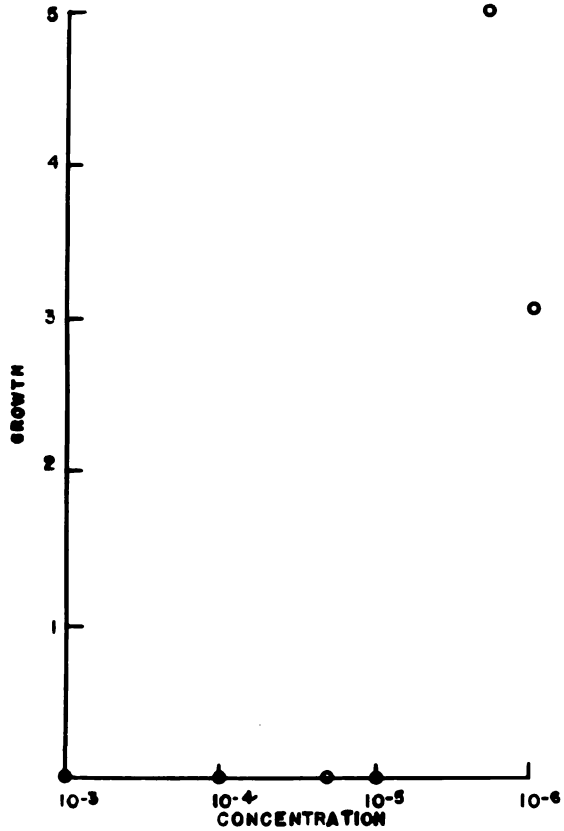

FIG. 19

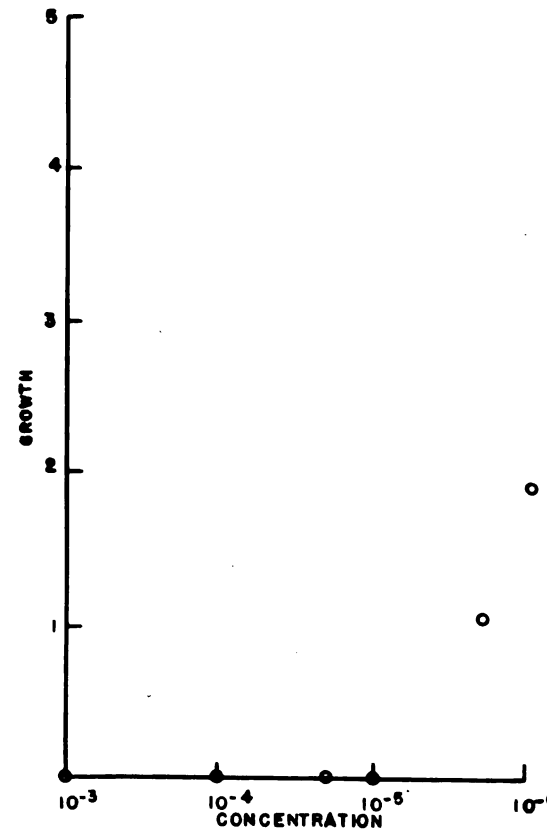

FIG. 20

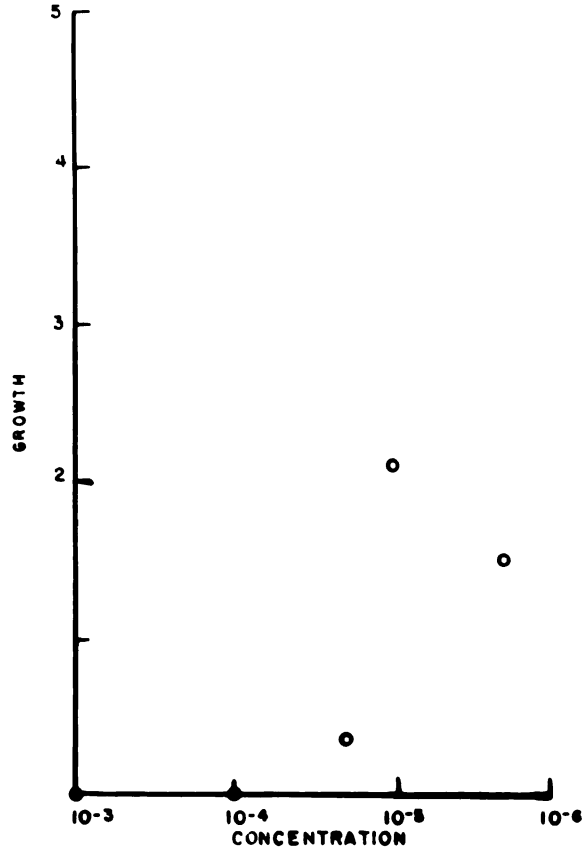

FIG. 21

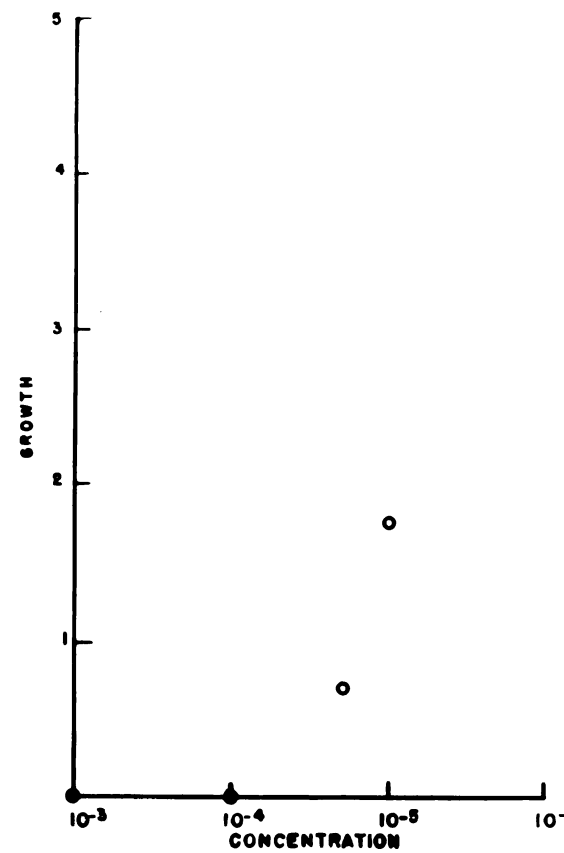

FIG. 22

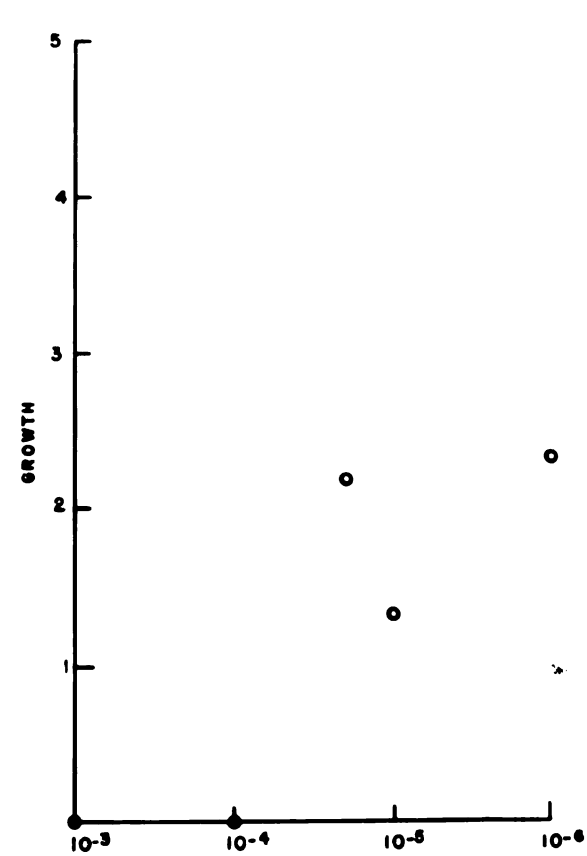

FIG. 23

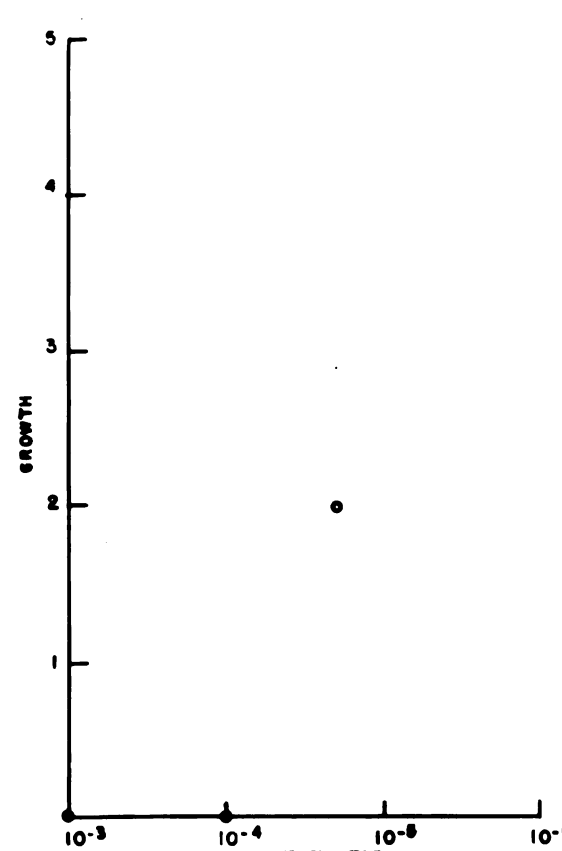

FIG. 24

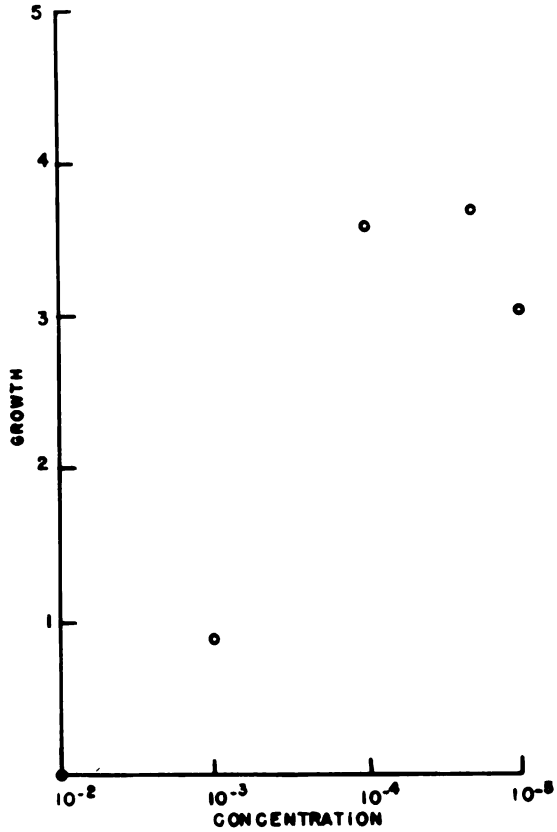

FIG. 25

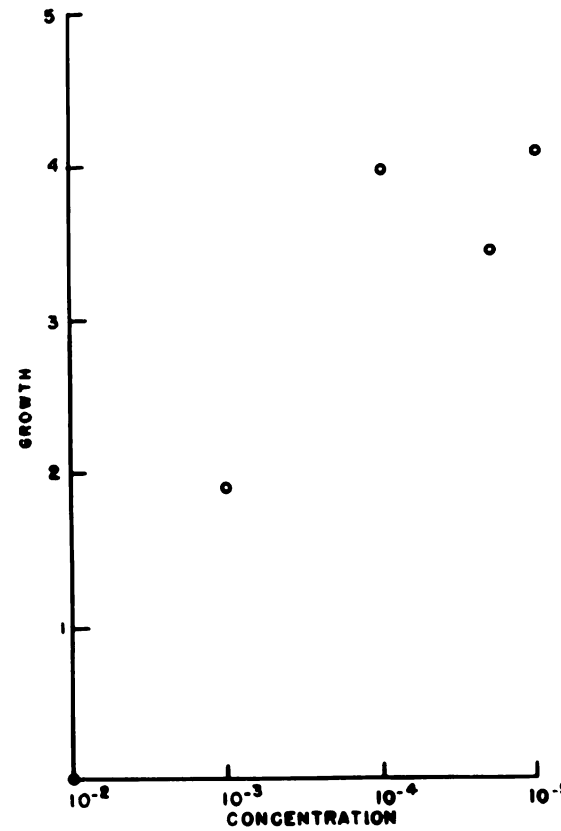

FIG. 26

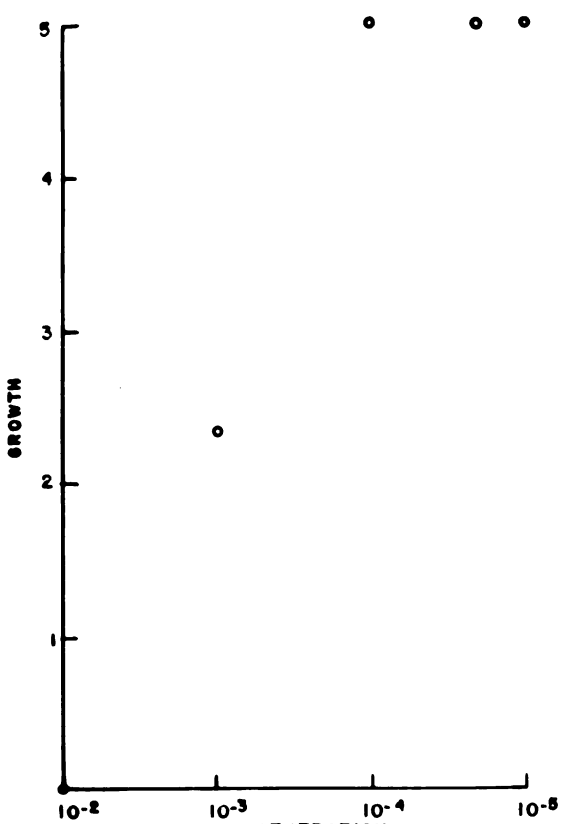

FIG. 27

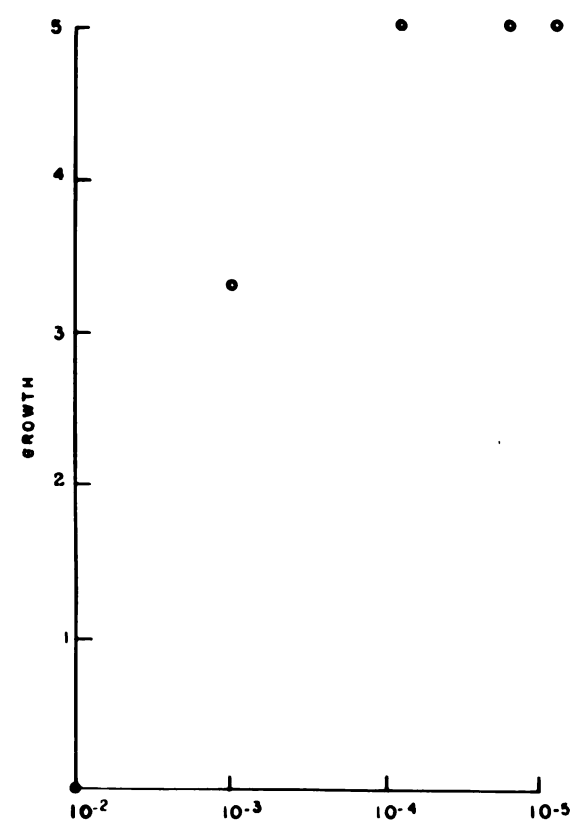

FIG. 28

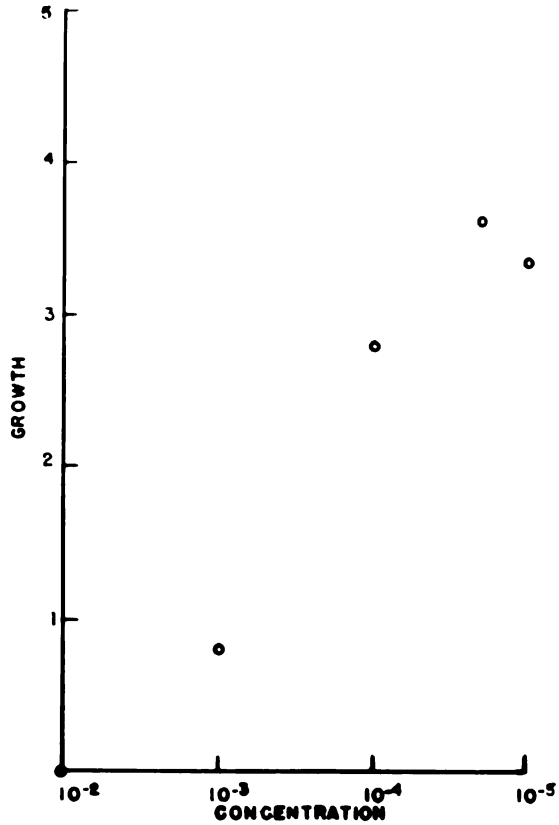

FIG. 29

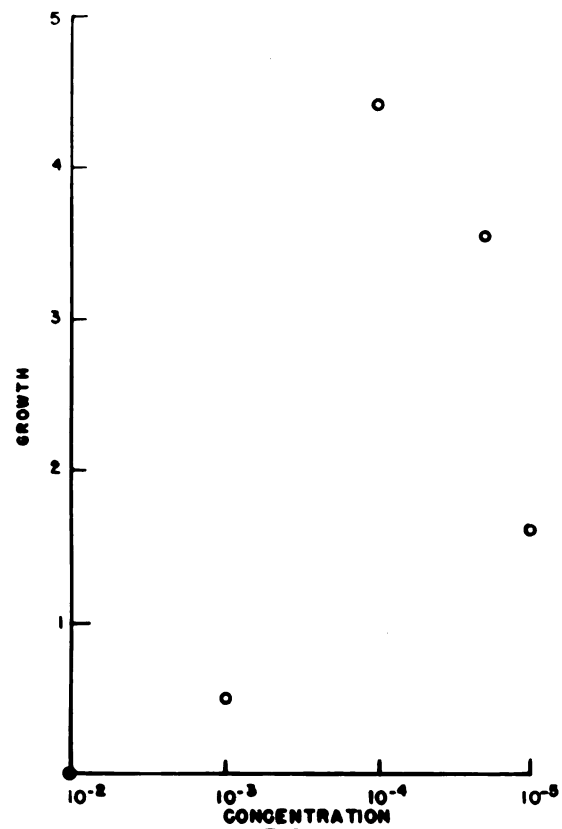

FIG. 30

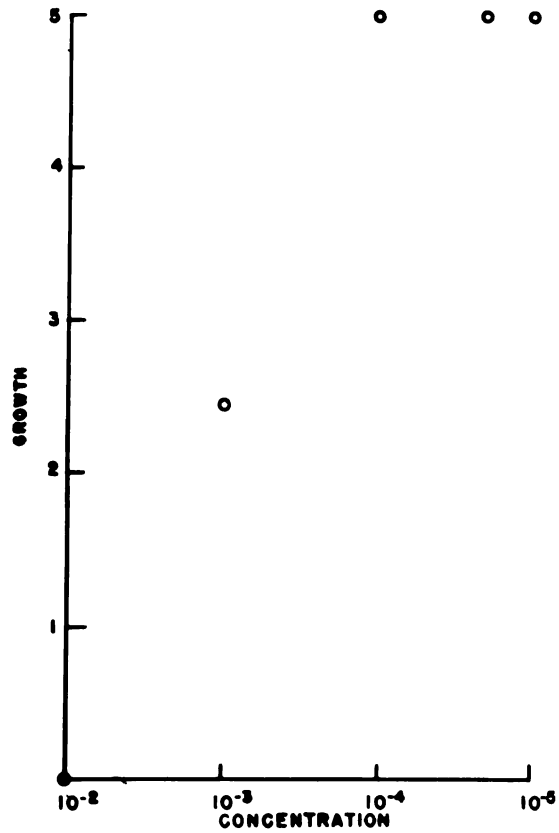

FIG. 31

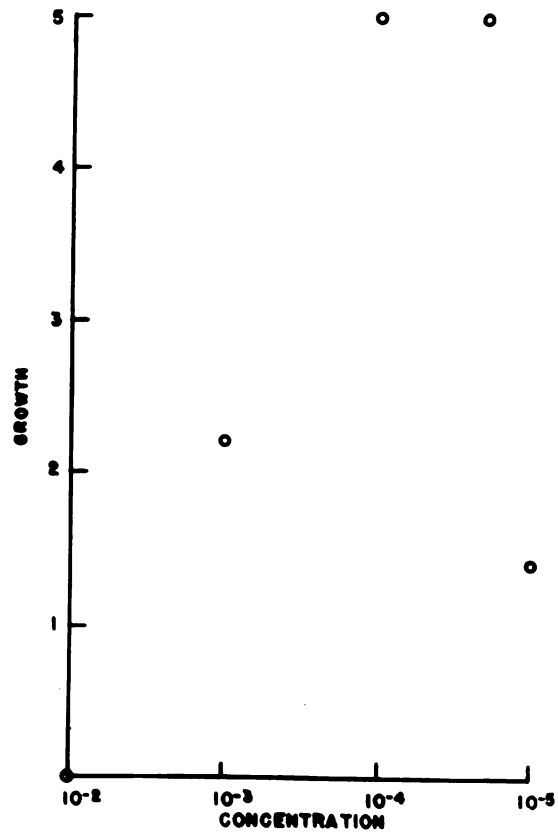

FIG. 32

Jan 30 5[illegible]