THE PHYSIOLOGY AND VARIATIONS OF CERCOSPORA BETICOLA IN PURE CULTURE

THESIS FOR DEGREE OF M. S.

FINLEY G. LARMER

1928

Fungi

Cercospora lythracea

THE PHYSIOLOGY AND VARIATIONS OF *CERCOSPORA BETICOLA* IN PURE CULTURE

Thesis presented in partial fulfillment

for the

Degree of Master of Science,

Michigan State College.

By

Finley G. Larmer,

1928

ACKNOWLEDGMENTS

The writer is indebted to Dr. G. H. Coons and Dr. E. A. Bessey for advice and suggestions given throughout this study, and furthermore to Dr. Coons for criticism and correction of the manuscript.

TABLE OF CONTENTS

The physiology and variations of Cercospora beticola in pure culture

Introduction

Cercospora beticola is a cosmopolitan fungus causing leaf spot of sugar beets (Beta vulgaris) and of allied plants such as the garden beet, mangold-wurzel, swiss chard, and other members of the family Chenopodiaceae. It is a common parasitic disease of the wild beet (Beta maritima) in Europe (8), and is sometimes found as a leaf spot disease on (Martynia Louisiana), a species of plant belonging to a family widely separated from the Chenopodiaceae. This plant is a native of the central and western plains states and is a common weed in the beet fields of Colorado.

The disease is evidenced as small brown spots, with reddish purple margins which later become ashen-gray at the center due to production of conidia. These spots are scattered irregularly over the surface of the leaf, but may become so numerous as to coalesce and cover a large portion of the surface, and may often cause a large portion of the leaf to die. In time with severe infections the leaf blade assumes a parched appearance, begins to discolor gradually from the distal portion toward the petiole and finally becomes brown, crisp and somewhat curled or rolled. The outer or older leaves are the first to show these effects and wither away after the petioles wilt. To replenish this deficiency of leaf area the plant produces

new leaves at the center of the crown. Consequently the crown becomes considerably elongated, and the root with early infections remains abnormally small because the food reserves of the plant are consumed in replacement of leaf tissue rather than for growth or storage. As a result of the high crown, wasteful topping must be practiced to eliminate certain organic salts that prevent the sugar from crystallizing after it has been extracted (18). This results in a two-fold loss, namely, reduction in sugar content and tonnage.

Cercospora leaf spot has long been recognized as being one of the most destructive foliage diseases of the sugar beet but its importance has in some beet growing areas been minimized because with certain rainfall distribution its damage is small. The fungus never causes complete destruction of the plant as is the case with many of the root-rot fungi, or the deformities of the foliage that characterize the virus diseases, especially curly-top. Infection by Cercospora beticola occurs in cycles thus enabling the host partially to recover by producing new foliage so that the severe effects of the disease on the growing plant are emphasized only in severe infections such as occur during wet seasons and in the irrigated regions of the west where rainfall and excessive use of ditch water form epidemic outbreaks.

Of the American workers, Pool and McKay (14,15) have contributed largely to our knowledge of the etiology of the disease. They have correlated epidemics of leaf spot with

climatic conditions, primarily the influences of temperature, humidity and other environmental factors on the production and dissemination of conidia. Aside from the reports of various experiment stations on the results of experiments for the control of the disease, there is little American literature dealing with Cercospora beticola.

This paper is a report of the results of studies of the reactions of the fungus to various cultural conditions, carried on during 1926-1927 at Michigan State College, and at the United States Department of Agriculture Field Laboratory at Rocky Ford, Colorado. The contents of the paper fall naturally into two sections, the first dealing with the morphology, cultural characteristics and physiological reactions to environment of the normal fungus, and the second dealing with the abnormal or variant forms that it has produced. The physiological studies were started in the fall of 1926, the work on variant forms being taken up the following fall. Infection experiments have been undertaken only in connection with the study of certain variant forms.

Morphology of the organism

Mycelium The mycelium formed in pure culture varies greatly according to the food constituents of the medium. The young hyphae are hyaline and tubular with thin walls and septae only at long intervals, and filled with a homogeneous finely granulated cytoplasm. As they grow older they become greenish-brown in color, build heavier walls and the cytoplasm

becomes coarsely granulated and filled with large highly refractive globular bodies which are soluble in ether. Septae are formed at short intervals and the mycelium often becomes gnarly, tortuous and irregular in shape. The aerial mycelium is usually more regular, somewhat smaller and less deeply colored, often almost hyaline. Sclerotium-like bodies composed of loosely massed hyphae are often observed in the submerged mycelium at the point of contact with the petri-dish. These bodies appear to be formed by abnormal division and growth of a few cells and are more prevalent in newly isolated cultures. They resemble very closely the sclerotium-like bodies formed in the substomatal spaces of the leaf from which the conidiophores arise. However, several of the masses have been isolated and kept in distilled water and both cultures but fructification has never been observed.

Conidiophores Fructification in pure culture results in abnormal conidiophores. Normal conidiophores are fasciculate, simple, erect and flavous, arising from a few celled stroma in the substomatal cavity of the leaf. Under field conditions, conidiophores are normally short and non-septate, although septation and elongation into hypha-like structures may occur in the older conidiophores (Plate I, Fig. 1). This latter condition is especially true on artificially inoculated plants grown under humid conditions in the greenhouse. The simple conidiophores may have one or two indistinct septae near the

base. They vary from 35 - 80 microns in length, and their average width is 4 - 5 microns. The older hypha-like conidiophores are sometimes as much as 180 microns long with septae 30 - 40 microns apart. In artificial cultures the younger conidiophores are short, simple and non-septate, arising directly from a submerged filament (Plate I, Fig. 1). At the apical end is seen a distinct scar, circular in outline, which indicates the point of attachment of the spore. Older conidiophores may have several of these scars along the surface with a geniculation at each scar. When the conidiophore is mature, and having borne a spore is put under favorable conditions, it sends out new growth from just beneath the scar. From this new growth a conidium is produced and abscissed leaving a distinct bend. The older conidiophores are long, septate and hypha-like, resembling the older structures found on plants. As the conidiophore begins to decline, conidia-like branches arise, these branches and the conidiophores serving as permanent hyphae (Plate I, Fig. 1).

Conidia Abnormal conidia varying from one or two-celled structures to extremely long multiseptate hypha-like filaments are sometimes produced (Plate I, Fig. 2). As a rule, conidia are typically obclavate to needle shaped, hyaline, multiseptate, and show typical circular attachment scars. Conidia from normal green cultures have a maximum length of 360 microns and a minimum length of 55 microns, the average length being 140 microns, and the average number of septae per conidium

being 10.8. The spore contents are finely granular, non-vacuolated and practically hyaline, conidia produced in pure culture being slightly more transparent than those produced on sugar beets.

Methods

Leaf spot material was obtained from the sugar beet growing districts of Colorado, California, Minnesota, Iowa, Nebraska and Michigan, all of which are more or less subject to severe epidemics of the disease.* Single spore cultures were obtained by the dilution plate method, the individual conidia being marked as soon as germination could be detected by microscopic examination. The germinated spore in a minute block of agar was transferred to corn meal agar in test tubes and furnished the stock cultures used in the experimentation. Cultures were identified by microscopic examination, cultural characteristics, production of conidia and in some cases by passage through plants, in which case the re-isolation was compared with the stock culture. Cultural studies have been made from both test tube and petri dish cultures, plate cultures being used most extensively since they are more easily observed.

Sugar beets used in artificial inoculation work were grown in pots in the greenhouse and inoculated by spraying spore and mycelial suspensions upon the leaves. The plants were then placed under bell jars and put in a temperature and

*See "Cultural strains of Cercospora beticola" in Supplement.

humidity chamber since moderately high temperature and humidity have been found to be favorable for infection.

Cultural characteristics

Many kinds of media have been used in the writer's study of Cercospora beticola since a careful delineation of the cultural characteristics of this organism on artificial media is needed to furnish ready means for its identification and in distinguishing the races or strains from one another. This work has also given information concerning conidial production and various physiological phenomena of the organism, and it will be indicated by the work to be presented that certain variants depart strongly from the mean behavior of the species.

Growth on media The fungus was found to grow well on most media employed. Plantings from corn meal agar cultures were made as shown in the following summary and the growth characters observed frequently but the results here given represent a summary of the observations and may be taken as the normal behavior of cultures which have grown for a ten day to two week period. The small mycelial threads are almost translucent but as they grow older the hyphae manufacture a pigment which varies in color with the character of the medium from a dark gray through the different shades of green to black, but most generally is an olivaceous green in the submerged and gray-green in the aerial mycelium as found on

corn meal agar. A reddish tint is sometimes produced with certain media, evidently due to some soluble pigment produced by the fungus. The following records were made after a ten day period from cultures growing under bell jars at room temperature under diurnal light conditions.

1. Corn meal agar: Submerged mycelium olive green, zoned, sclerotium-like bodies; aerial mycelium greenish-gray, abundant, fluffy, somewhat clumped. Diam. 19 m/m. No typical spores.

2. Oat meal agar: Submerged mycelium pea green, shallow, zoned; aerial mycelium sparse, white tuft in center. Diam. 18 m/m. A few typical spores.

3. Potato dextrose agar: Mycelium almost black, shallow in substratum, zoned faintly, exuding amber colored fluid; aerial mycelium profuse, variously colored, gray background, felty and matted. Diam. 17 m/m. No typical spores.

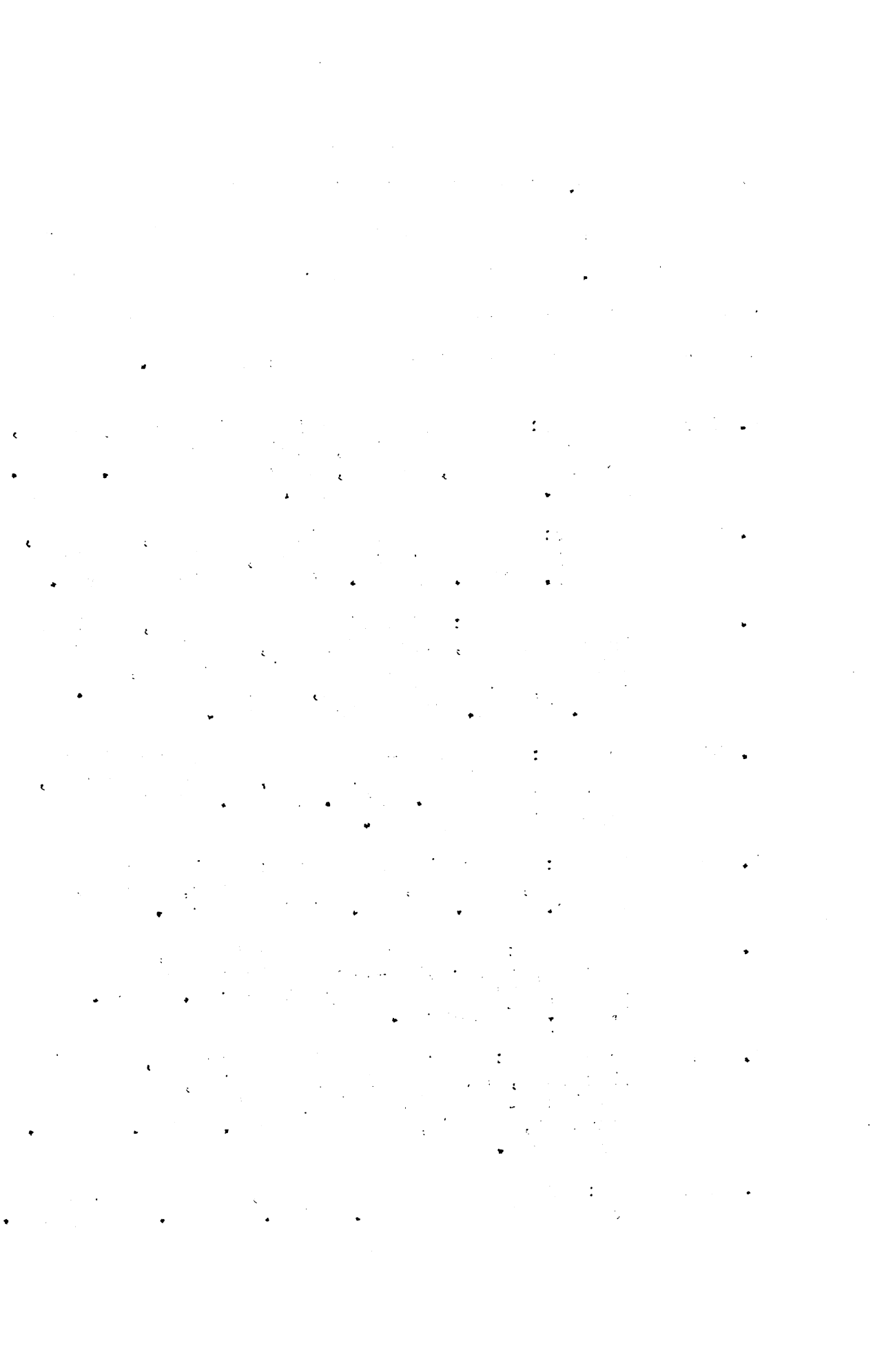
4. Lima bean agar: Greenish-black submerged mycelium; aerial mycelium heavy, felty, black at border, white in center. Diam. 21 m/m. Typical spores in new growth.

5. Bean pod agar: Black in substratum; aerial growth abundant, cottony, white in center, dark at border. Diam. 17 m/m. A few spores.

6. Prune juice agar: Deep green in substratum, zoned faintly, sclerotium-like bodies; aerial growth sparse, white and fluffy at center. Diam. 24 m/m. No spores.

7. Malt extract agar: Dark green in substratum, mycelium distorted, full of globular bodies, dispersed reddish pigment in medium; aerial mycelium abundant, fluffy, white to gray. Diam. 25 m/m. No spores.

8. Plain agar: Submerged growth sparse, pale green; little or no aerial mycelium. Diam. 22 m/m. No spores.



9. Neutral red agar: Growth shallow and tufted; aerial mycelium short, grayish-black. Diam. 7 m/m. Numerous short irregular spore-like structures, germinated like conidia.

10. Litmus lactose agar: Black in substratum; grayish-black, low, felty aerial growth. Diam. 18 m/m. No spores.

11. Endo's agar: Mycelium in substratum dark, takes up red pigment; aerial mycelium short, grayish-brown. Diam. 15 m/m. No spores.

12. Coon's synthetic agar: Rich green in substratum; aerial growth abundant, felty, uniformly grayish-white. Diam. 25 m/m. No spores.

13. Nutrient agar: Submerged mycelium green, amber colored fluid exuded through grayish-white, felty aerial mycelium. Diam. 15 m/m. No spores

14. Patel's crystal violet agar: Greenish-brown in substratum; gray, felty aerial mycelium. Crystal violet reduced to yellow by growth of the fungus. Diam. 13 m/m. No spores.

15. Raw vegetable media: Potatoes, carrots, sugar beets, garden beets, turnips and sweet potato plugs in sterile test tubes - no growth. Beet leaf petioles in test tubes - slight grayish aerial mycelium at point of inoculation, slight penetration of tissues, epidermis must be broken for growth.

16. Bouillion broth: Inoculum placed on surface. Surface growth gray and felty. Submerged mycelium hyaline and irregular. No spores.

17. Nutrient broth: Characteristic grayish-green growth at surface of liquid. No spores.

18. Sugar solutions: Sucrose, dextrose, mannnse, maltose, levulose, galactose and glycerine in 1.0% solutions in sterile distilled water. Inoculum placed on filter paper cones in preparation dishes. No growth.

19. Nutrient sugar agars: Made up from nutrient broth to which 1.0% sugar and 1.0% agar agar had been added.

Check - growth pellicled, black. Levulose and glycerin - same as the check. Mannose - greenish-gray, felty and matted. Diam. 14 m/m. Galactose - deep greenish-gray, felty and matted. Diam. 15 m/m. Dextrose - growth felty, light gray to gray. Diam. 30 m/m. Maltose - more fluffy, less matted, greenish-gray. Diam. 22 m/m. Sucrose - similar to maltose, slightly lighter in color. Diam. 30 m/m. No spores found on any of these media.

20. Nutrient sugar agars with dyes: Brom cresol purple - tolerated by the fungus with a variable degree, slight indication of reduction of dye on sucrose, dextrose, maltose, levulose and galactose. Brom thymol blue - intolerant to the dye, growth very slight on all sugars.

21. Beet leaf broth: Growth felty in pellicle at surface, black. A few atypical spores.

22. White rice: Mouse gray, olive green at points of contact with test tube. No spores.

23. Castor bean seeds: Mycelium intensely black, full of oil globules. No spores.

24. Corn grains: Mycelium black with short greenish-gray felty aerial growth. No spores.

25. Lima bean seeds: Mycelium greenish to black; short velvety mouse gray aerial growth. No spores.

26. Peas: Very similar to lima bean seeds, slightly darker and more velvety. No spores.

27. Sunflower seeds: Similar to growth on castor bean seeds. No spores.

28. Potato plugs: Tough black growth in substrata; cottony gray aerial mycelium. No spores.

29. Carrot plugs: Mouse gray to white aerial growth; greenish-black mycelium in substratum. No spores.

30. Beet leaf petioles: Olive green in substratum; ashen gray fluffy aerial mycelium. A few atypical spores.

31. Beet leaf agar:* Greenish-black in substratum, distinct zones; aerial mycelium sparse, low, mealy, deep green. Poor vegetative growth. Abundant typical spores.

Corn meal agar has been found to be the best differential medium used in these studies of Cercospora beticola. The fungus produces a characteristic olive green colony** with greenish-gray aerial mycelium. Alternaria sp. is sometimes obtained as a contamination in dilution plate isolations of Cercospora beticola from the sugar beet leaves. In color and appearance of submerged growth it resembles Cercospora beticola but is readily recognized by the conidia which are very abundant on corn meal agar. Beet leaf agar has been of value in studying conidia production in pure culture, but is not a good nutrient medium for vegetative growth.

Influence of depth of medium and amount of nutrients

In order to determine the effects of the depth of medium and quantity of nutrients upon the organism the following tests were carried out. Petri dishes were supplied with 12 cc. and 25 cc. and deep culture dishes with 50 cc. of the nutrient agars listed in Table I and inoculated with strain C-8.

*Preparation of beet leaf agar:

1. Young sugar beet leaves dried at 40° C. and finely powdered.
2. 15 grams of beet leaf flour and 15 grams of agar agar cooked slowly in distilled water for 30 minutes.
3. Strained through cheese cloth and made up to 1000 cc.
4. Autoclaved at 15# pressure for 30 minutes.

**Following general usage in plant pathology, the so-called "pseudo-colony" of a fungus is termed a "colony".

Parallel cultures placed under similar light and temperature conditions under bell jars gave such similar results as to make separate records unnecessary. As will be seen from the table, differences in linear growth on plates of different depth of medium were practically negligible at the end of a ten day period but favored the 25 cc. plates after twenty days, with the 12 cc. plates showing the least growth.

Table I

Influence of depth of medium on linear growth of C. beticola

Agar medium	12cc.		25 cc.		50 cc.	
	10 days	20 days	10 days	20 days	10 days	20 days
Oat meal	17	62	18	63	18	70
Litmus lactose	19	27	18	49	18	50
Potato dextrose	20	70	19	69	22	72
Bean pod	19	64	18	70	21	70
Corn meal	20	62	20	76	20	69
Malt extract	22	67	25	82	19	80
Beet leaf	19	55	18	48	18	42
Plain	17	61	18	69	18	66
Coon's synthetic	25	82	25	80	23	80
Neutral red	12	24	10	36	11	36
Prune juice	20	74	21	76	19	68
Lima bean	21	71	17	68	19	76
Endo's	14	39	14	45	15	45
Average	19.6	59	18.5	64	18.5	62

(Values represent diameter of colony growth in millimeters.)

Since the cultures were grown on solid media there was no accurate method of weighing the actual amount of mycelial growth so observations were made on the density and amount of

aerial growth and the depth to which the submerged mycelium had penetrated the substratum at the end of the experiment. At this time the growth had attained the greatest depth in the 50 cc. dishes and the aerial mycelium was profuse and matted. There was a general tendency for the aerial mycelium to become lighter in color as further growth occurred. From the experiment the conclusion can be drawn that the amount of nutrient present affects the growth materially after the first ten days in that an abundance of food permits further growth of the mycelium formed during the initial period of growth. The 12 cc. plate cultures had probably consumed the available nutrients during the initial growth and the fungus had entered a period of quiescence until more food was available. Depth of medium probably affects the cultural characteristics only in so far as the quantity of nutrients is concerned.

Light relations Since many organisms react in a specific manner to light stimulus, the following tests were carried on to determine the effects of light upon various strains of Cercospora beticola. Strains C-11, C-13 and C-23 were planted in petri dishes containing 15 cc. of corn meal and potato dextrose agars and kept under the different conditions of light as indicated in Table II.

Table II

Growth of Cercospora beticola in relation to light

Condition of growth	Temperature	Medium	Strain	Colony diam. m/m Days of growth 2	6	10	14
Darkness	22°-24° C.	Potato dextrose agar	C-11				42
			C-13				45
			C-23				43
		Corn meal agar	C-11				26
			C-13				25
			C-23				27
Ordinary daylight conditions	21°-23° C.	Potato dextrose agar	C-11	2	13	24	36
			C-13	2	17	31	42
			C-23	2	15	26	38
		Corn meal agar	C-11		9	17	23
			C-13		11	20	27
			C-23		12	20	26
Light from 100 watt nitrogen filament bulb	24°-25° C.	Potato dextrose agar	C-11	2	16	29	40
			C-13	2	16	27	38
			C-23	2	14	25	36
		Corn meal agar	C-11		11	21	25
			C-13		10	19	24
			C-23		11	20	24

It is evident from the records given in the table that light relations are not very marked on the growth of Cercospora beticola. These findings are similar to those of Klotz (11) in respect to Cercospora apii, a closely related species. Linear growth is slightly more rapid in darkness than it is in either light or diurnal conditions of light but this difference is so small that it is probably negligible. Growth on corn meal agar was abundant, and matted in all forms under the different conditions, differing only in color of mycelium which was grayish-white under continuous light, a light greenish-gray under diurnal light and a dark greenish-

gray in continuous darkness. Growth was more rapid on potato dextrose agar, being abundant and felty, but the color contrast was lacking, all cultures having light greenish-gray aerial mycelium. Observations made at different times showed only a difference in color of mycelium between cultures grown in light and darkness, cultures in light being somewhat lighter colored.

Sporulation in some fungi has been found to be a direct response to light stimulus. Coons (7) found that pycnidia were produced by Plenodomus fuscomaculans only when cultures were grown in light. Hedgecock (10) determined that spores were produced in darkness in artificial cultures of Cephalothecium roseum and other fungi, while Gallemaerts according to Bisby (1) found that light inhibits mycelial growth and thus stimulates sporulation in the same fungus. Leonian (12) found that light was required for the production of pycnidia in species of Sphaeropsidales. According to Bisby (1), Molts reports that conidial cushions are almost entirely absent in cultures of Sclerotinia fructigena when grown in darkness, and himself concludes that light affects the tips of the hyphae giving them a brief check or resting period resulting in conidia formation.

To study the effect of light on conidia production, strains, C-11, C-13 and C-23 were planted on 18 cc. of beet leaf agar in 250 cc. flasks and kept under the conditions described in Table II when the data were taken.

Table III

The effect of light on conidia production in C. beticola

Strain	Light 24-25° C.		Diurnal 21-23° C.		Darkness 22-24°C.	
	Growth	Spores	Growth	Spores	Growth	Spores
C-11	34	++ typical	33	+++ typical	37	++ typical
C-11	35	do	31	do	37	do
C-13	36	do	33	do	34	do
C-13	36	do	33	do	35	do
C-23	34	do	34	do	35	do
C-23	34	do	34	do	34	do
Av.	35.8		33.3		35.3	

(Numerical values represent diameter of colony growth in m/m.)

Conidia were produced under all conditions of light and darkness but cultural washings indicated that they were produced most numerously under diurnal conditions. This will probably explain the slightly slower linear growth as being due to checking of the mycelial filaments resulting in conidia formation under fluctuating light.

Temperature relations The occurrence of epidemics of Cercospora leaf spot during the late summer months would indicate that high temperatures are necessary for its optimum development. A nine compartment differential therm-regulator was employed in studying the relation of temperature to growth of the organism. The low temperatures were obtained by keeping ice packed in one end and the high temperatures by the use of a small electric heater which was placed in the opposite end. This gave a series of fairly constant temperatures varying by steps from 10-40° C. Inoculum from corn meal agar cultures of

strains C-11, C-13 and C-23 was transferred in duplicate to 15 cc. of agar in petri dishes and incubated for a period of ten days, when the data were taken. As there were only slight differences between strains in a given medium, the data have for simplicity and convenience been averaged and recorded collectively for each temperature range.

Table IV

The effects of temperature on growth of C. beticola

Comp.	Temp. range	Medium agar	Diam. of Colony m/m Max.	Av.	Min.	Character of growth
1.	6-10°C	Corn m.	-	-	-	Too slight to measure
		Nutrient	-	-	-	do
		Pot.dex.	-	-	-	do
2.	14-16°C	Corn m.	7	6	5	Poor, deep olive green
		Nutrient	11	9	4	do
		Pot.dex.	15	10	4	do
3.	17-18°C	Corn m.	16	14	13	Fair, green, aerial slight
		Nutrient	18	15	13	do , mouse gray, do
		Pot.dex.	19	17	15	do , greenish gray, do
4.	19-21°C	Corn m.	20	18	16	Good, deep olive green
		Nutrient	18	17	13	do , dark gray
		Pot.dex.	26	24	22	do , greenish-gray
5.	22-23°C	Corn m.	18	15	14	Like (4)
		Nutrient	16	15	14	do
		Pot.dex.	28	26	24	do , slightly better
6.	24-26°C	Corn m.	28	23	20	Optimum, like (4) in color
		Nutrient	18	16	14	do
		Pot.dex.	30	28	27	do
7.	27-29°C	Corn m.	32	25	20	Profuse, pale growth
		Nutrient	15	15	14	do , white aerial growth
		Pot.dex.	32	30	30	do , white and felty
8.	32-34°C	Corn m.	14	9	8	Poor, aerial pale, heavy
		Nutrient	7	6	6	do , white throughout
		Pot.dex.	15	9	3	do , aerial white, heavy
9.	37-40°C	Corn m.	-	-	-	No growth
		Nutrient	-	-	-	do
		Pot.dex.	-	-	-	do

These data indicate that the fungus grows well between 20° and 30° C., temperatures above or below these limits being

detrimental to the best growth of the organism. The optimum temperature as determined by the size and color of the mycelium is around 24-26° C. Good growth takes place below the optimum but with a gradual decline in rate of growth. Temperatures above the optimum seem to stimulate abnormal growth, the organism becoming pale and producing considerable aerial mycelium.

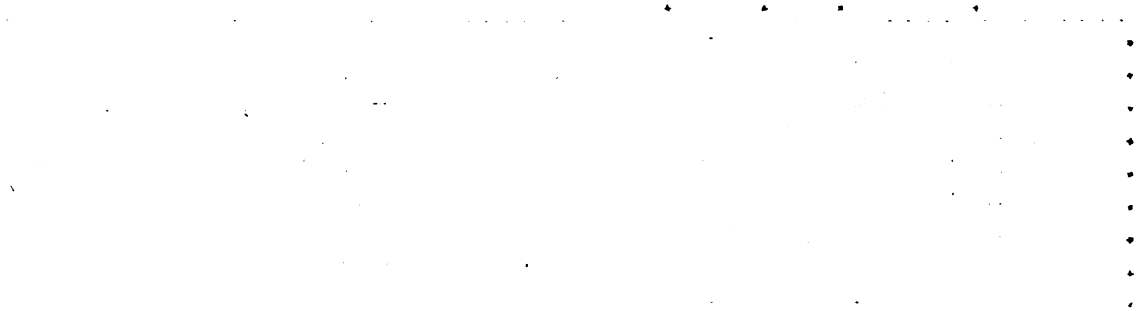
Pool and McKay (14) report that conidia production on the host plant is greatly influenced by temperature and humidity. Humidity is apparently the determining factor since sporulation occurs over a wide range of temperature but only under conditions of high humidity. However, heaviest sporulation occurs at temperatures at the lower limits for optimum growth of the organism, which was between 24-29° C. on beet leaf agar, slightly higher than for ordinary culture media.

Duplicate transfers of strains C-11, C-13 and C-23 were made on beet leaf agar in petri dishes and inoculated at the various temperatures of the thermo-regulator for ten days.

Table V

The effects of temperature on conidia production in cultures of Cercospora beticola

Comp.	Temp. (°C.)	Colony Diam. m/m Max.	Av.	Min.	Character of growth	Spores
1.	6-10	-	-	-	None	None
2.	14-16	7	6	5	Poor	None
3.	17-18	14	13	11	Fair	+ short, robust
4.	19-20	20	18	16	Good	++ typical
5.	22-23	20	18	13	Good	+++ typical
6.	24-26	23	19	15	Optimum	+++ longer than in (5)
7.	27-29	22	19	15	Abnormal	+ few and long
8.	32-34	14	10	5	Poor	0 few and hypha-like
9.	37-40	-	-	-	Death	None



Formation and germination of conidia Conidia production on artificial media has never been reported in any of the literature reviewed, although it has been known to occur in other members of the genus Cercospora (9,11). Typical conidia have been found on beet leaf agar and more or less normal spores have since been found on several media. However, such instances are rare and the conidia are so abnormal that they could be readily overlooked. Conidiophores and conidia have been described and compared with those produced on plants under "Morphology".

Spores are produced over the entire surface of the mycelial growth but they are seen in greatest numbers at the borders of the colony, due probably to the formation of hypha-like conidiophores and the abscissing of the conidia. Apparently light and temperature play minor rôles in sporulation, conidia being produced over a temperature range of 17-39° C. with the heaviest spore production occurring at about 25° C. which is the lower limit of temperature for optimum growth. Humidity with proper nutrients is probably the determining factor, heaviest sporulation taking place at 60% humidity (14) with very few conidia being produced in a dry atmosphere. High temperature stimulates linear growth, conidia often exceeding 360 microns under these conditions. Short robust spores with few septae are characteristic of low temperatures.

Conidia produced in artificial cultures germinate in the usual manner (Plate I, Fig. 3). The end cells germinate first,

sending out long slender hyaline sometimes branching germ tubes, and then shortly afterwards the intermediate cells of the conidium germinate. It is seldom that more than 50% of the conidial cells germinate, dessication of the non-viable cells apparently taking place. Infection on sugar beets can be produced as readily with these conidia as it can be with conidia produced on the plant.

Heavy fructification on beet leaf agar is indicated by a dark olive green to black mycelium in the substratum, with sparse aerial growth which is dark, short and mealy like. There is a tendency for cultures to lose their power of conidia production when grown in artificial cultures continuously for long periods. Loss of vegetative vigor and color are generally indicative of partial sterility. Achromatic cultures resulting as gradual or sudden variations are invariably sterile although conidia much smaller than normal have been observed in a few instances. Heavy aerial mycelium is usually accompanied by low conidia production.

From the data given the conclusion can be drawn that conidia production in artificial cultures of Cercospora beticola is a response to a food stimulus influenced more or less by other factors such as humidity, light and temperature.

Loss of color Loss of the characteristic green color through reduction in the amount of greenish-brown pigment in the mycelium has occurred commonly when the fungus has been kept in culture on artificial media for long periods of time. Factors which seem to influence this phenomenon are high temperatures,

dessication in old cultures and quality and quantity of nutrients. None of the factors when tried experimentally has induced permanent loss of color. Cultures have been repeatedly grown at high temperatures resulting in pale colored, almost achromatic growth which immediately became normal green when transfers were made from these cultures and grown under normal conditions.

Observations on the effects of dessication in relation to loss of color in subsequent transfers were made by taking inoculum from normal olive green cultures that had dried out with age. The following table shows the results of transfers made from the original stock culture of several forms, over different periods of time. Transfers were made on corn meal and potato dextrose agar slants and incubated at 24° C. for ten days before observations were made.

Table VII

Viability of C. beticola in cultures

Strain	Age of culture	Transfer of 12/3/37		Transfer of 3/30/38	
		Corn meal agar	Pot. dex. agar	Corn meal agar	Pot. dex. agar
C-1	10/31/36	Normal	No growth	Normal	Normal
C-2	10/31/36	do	Normal	do	do
C-3	10/31/36	do	do	do	do
C-4	11/1/36	do	do	do	do
C-5	11/1/36	do	do	do	No growth
C-10	11/5/36	do	do	do	Normal
C-11	11/5/36	do	do	do	do
C-13	11/5/36	do	do	do	do

These data show that only normal cultures were obtained from eighteen month old cultures that were completely dried out.

Subtransfers from these cultures after they had grown for thirty days gave only normal forms with no indication of loss of color.

Growth on certain media produces cultures that are pale green in color; this being particularly true of a 1.2% washed plain agar medium. Strains C-6 and C-17 were grown on plain agar medium and small quantities of inoculum were taken from the edge of the colony to obtain as near a homogeneous culture as possible. This inoculum was transferred to petri dishes containing 15cc. of plain agar medium and allowed to grow for fifteen days when the cultures received the treatments indicated in Table VII.

Table VII

Treatment of pale cultures with amino acids

Treatment	Strain C-6	Strain C-17
Leucine	No change	Same as C-6
Tyrosine	Greenish-black	do
Alamine	New growth light green	do
Glycine	No change	do
Cystine	do	do
Asparaginic acid	New growth normal green	do
Corn meal agar	Normal culture	do
Check	No change	do

This experiment was run in duplicate and repeated with similar results. It indicates that amino nitrogen is available and has a tendency to bring out the green pigment. Achromatic cultures of unknown origin and the aberrant form of strain C-1 have been treated similarly with negative results.

These data indicate that the abnormally pale forms studied are not of the same nature as those which are obtained either through continuous culture or the aberrant forms, in that they immediately return to the normal green color when grown under favorable conditions.

Zonation

The formation of zones, i.e., production of concentric rings of light and dark mycelium, is a peculiarity exhibited by many fungi growing in artificial cultures. Structural characteristics of the colony growth such as varying density and amount of spore massing, grouping of pycnidia and sclerotia, mycelial branching, color, etc., due to differential growth are largely responsible for the occurrence of this phenomenon. These effects have been attributed to various casual agencies such as light relations, temperature relations, resting periods, staling products, mycelial crowding, alkaline medium and variations in the amount of nutrients. Stevens and Hall (17) emphasize the fact that zonation may be induced by fluctuation of the above external factors but that it is largely dependent upon the fungus.

Zonation was first observed in test tube cultures but later was found to be very pronounced in petri dish cultures. By correlating the number of bands with the age of the colony it could be seen that the phenomenon was in some way associated with diurnal fluctuations. Linear growth is normally slow in

Cercospora beticola, consequently the bands are narrow and often indistinct. This together with the fact that zonation occurred in cultures in which there were no conidia formed and was not due to fructification on beet leaf agar, instigated experiments to determine the characters involved and the causative factors responsible for the expression of this differential growth.

Types of zonation Several types of zonation have been observed, the most common being due to the differential rate of linear growth of the submerged mycelium. In this type the aerial mycelium is often felty, showing a uniform color and texture throughout the colony, and can be removed without destroying the concentric rings as they are in the submerged mycelium only. Microscopic examination shows that the dark bands are due to a large number of mycelial threads and the light bands to a small number of mycelial filaments. It seems that in this fungus the dense crowding of the filaments resulting from their repeated branching inhibits growth, resulting in a period of quiescence, followed by the onward growth of a few of the more vigorous scattered hyphae. This process is repeated indefinitely, the rapidity of succession of zones being dependent upon the relation existing between the rate of branching and increase in linear growth which is controlled by fluctuating external conditions. The mycelial filaments in the dark bands are irregular and somewhat distorted and twisted, while the filaments in the lighter bands are smooth,

even and regular. Associated with this type we often find aerial growth appearing in concentric rings which coincide with the zones in the submerged growth. This type is especially prominent on lima bean agar but has not been associated with sporulation but rather with clumping of the aerial mycelium. Occasionally cultures produce a red pigment in the medium, which often occurs in distinct regions or zones.

Light relations That zonation in artificial cultures of certain fungi is a direct effect of fluctuating light conditions has been conclusively proven by several investigators. However, a general rule for the specific effect on all fungi cannot be made. Then too, density and grouping of fruiting bodies have been the characters most commonly associated with the formation of zones. Since spores are not produced in cultures of Cercospora beticola on standard media, no certainty could be placed on the influence of light without observing its effects under conditions where other factors were controlled. In conjunction with the experimental work on relation of light to zonation, observations were made on the effects of red and blue wave lengths since Miss Parr (13) has shown that Pilobulus gave varying phototropic responses for different portions of the spectrum. Hedgecock (10) found that blue light was comparable to daylight and red light to darkness in his studies of Cephalothecium roseum. According to Bisby (1), Gallemaerts working with the same genus obtained contradictory results in that red, orange and blue lights all gave the same reaction as diffused daylight.

Experimental data Data on zonation of Cercospora beticola in relation to fluctuating light conditions and different wave lengths of light are given in Table VIII, page 27. Culture dishes of the same diameter and depth were used throughout the experiment. Twenty cubic centimeters of agar were poured into each petri dish, thus assuring the same volume and depth of medium and the same atmospheric conditions for all cultures. Eight different media and four different strains of Cercospora beticola were used, inoculum being placed in the center of each quadrant of the petri dish. The cultures were incubated in special chambers designed for studying light relations. The temperature was found not to vary beyond the limits of 32-34° C. at any time that readings were taken. All joints in the chambers were sealed with modeling clay, thus assuring that light was admitted only through the glass filters. Diffused daylight was admitted through a north exposure, and the red and blue lights were obtained by passing natural light from the same source through glass filters which cut out all except the red and blue lights respectively. Continuous light was furnished from a 110 v.-100 w. nitrogen filament lamp, as was the light for the 10 and 30 minute exposure. Observations on zonation were made after the cultures had grown for twelve days.

The exposure of cultures of Cercospora beticola to diffused daylight alternating with darkness caused zonation in all cultures on all media except plain agar, and no zonation was

Table VIII

Effect of light on zonation in C. beticola

Agar medium	Diurnal darkness blue light				Diurnal darkness red light				Diurnal conditions				Continuous light				Continuous darkness				Darkness with light exposures of 10 min.				Darkness with light exposures of 30 min.			
	1	8	20	27	1	8	20	27	1	8	20	27	1	8	20	27	1	8	20	27	1	8	20	27	1	8	20	27
Corn meal	+	+	+	+	0	0	0	0	+	+	+	+	0	0	0	0	-	-	-	-	-	-	-	-	+	+	+	+
Pot. dex.		+	+	+	0	0	0	0	+	+	+	+			0	0	0	0	0	0		0	0	0		+	+	+
Beet leaf	+	+	+	+	0	-	0	-	+	+	+	+		0	0	0	-	0	-		-	-	-	-	+	+	+	+
Oat meal	+	+	+	+	0	-	0	-	+	+	+	+	0	0	0	0	-	-	-	-	+		+	+	+		+	+
Lima bean	+	+	+	+	0	0	0	0	+	+	+	+	0	0	0	0	0	0	0	0	0		0	0	+		+	+
Malt ext.	+	+	+	+	0	0	0	0	+	+	+	+	0	0	0	0	0	0	0	0	0		0		+		+	
Prune juice		+	+	+	0	0	0	0	+	-	+	+	-	0	0	0		0	0	0		0	0	0		+	+	+
Plain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 No zonation
- Zonation very faint
+ Zonation pronounced

observed on this medium under any condition. However, due to density of the aerial mycelium the zones were somewhat marked on some media. Concentric rings were especially prominent on corn meal, oat meal and beet leaf agars on which the fungus produces only a moderate amount of aerial mycelium in twelve days. Allowing two days for the fungus to start active growth, zonation was correlated with diurnal fluctuations, there being ten distinct rings formed over a twelve day period. Blue light alternating with darkness gave comparable results with daily fluctuations while on the whole zonation was not caused by red light. Very faint traces of zonation were observed in some cultures exposed to red light as was the case in cultures kept in constant darkness. Cultures kept in continuous light gave no evidence of zonation. Distinct growth bands were induced on oat agar and faint bands on corn meal and beet leaf agars by exposing cultures for 10 minutes but a 30 minute exposure was required to cause zonation in cultures growing on the other media.

The 10 and 30 minute exposures were made every two days, the growth of the colony being marked each time. The narrow dark bands were found to be produced beneath the marks indicating the point of growth at the time of exposure to light, and the wide bands of light colored mycelium were produced during periods of darkness. This indicates that light has an inhibiting effect on the hyphae at the growing tips, causing profuse branching to form the dark growth bands.

Temperature relations Investigators disagree as to the part played by fluctuating temperatures in causing zonation. Chaudhuri (4) concludes that there is no correlation between periodic external conditions and zonation. He found that cultures of Verticillium albo-atrum produced zones in constant darkness only at 35° C., but that zonation was evident in cultures grown in constant light at 31-33° C., indicating that different conditions of light and temperature influenced zonation but that the phenomenon itself was induced by other causative factors. Hedgecock (10) was unable to attach any significance to fluctuating temperatures in relation to zonation in Cephalothecium roseum and other fungi. Conversely, Bisby (1) reports that temperature plays an important part in the zonation phenomenon. He shows that zonation was induced in Fusarium sp. grown in constant darkness by alternating temperatures between limits of 10-25° C. He attributes this to differential growth of mycelium and not entirely to conidia production. Substantiating this, Christensen (6) produced distinct bands in cultures of Helminthosporium sativum by alternating temperatures in both constant light and darkness.

Using the same type of petri dishes and media prepared at the same time as was used in the light experiments, an attempt was made to determine the relationship of fluctuating temperatures and zonation in cultures of Cercospora beticola. The same four strains were planted as in the previous experiment and all petri dishes were carefully wrapped to exclude light.

Set (A) was kept in an incubator at 24° C., Set (B) was alternated between temperatures of 24° C. and 12° C. every 48 hours, and Set (C) was kept at a constant temperature of 12° C. in a chamber of the ice box.

Table IX

Temperature in relation to zonation in cultures of C. beticola

Agar Medium	24° C. Constant				12-24° C. Alternating				12° C. Constant			
	Strain C-				Strain C-				Strain C-			
	1	8	20	27	1	8	20	27	1	8	20	27
Corn meal	0	0	0	0	+	+	+	+	0	0	0	0
Potato dextrose	0	0	0	0	+	+	+	+	0	0	0	0
Beet leaf	0	0	0	0	+	+	+	+	0	0	0	0
Oat meal	0	0	0	0	+	+	+	+	0	0	0	0
Lima bean	0	0	0	0	+	+	+	+	0	0	0	0
Malt extract	0	0	0	0	+	+	+	+	0	0	0	0
Prune juice	0	0	0	0	+	+	+	+	0	0	0	0
Plain agar	0	0	0	0	+	+	+	+	0	0	0	0

The experiment was repeated using only corn meal and oat meal agars but using more culture forms. Set (A) and (C) were kept as before but Set (B) was given different lengths of exposures to the various temperatures, i.e., five days at 24° C. and twelve hours at 12° C; five days at 24° C. and twenty-four hours at 12° C. and then five days at 24° C. before the observations in Table X were made.

Alternating temeratures produce distinct growth bands, low temperatures inhibiting growth to such an extent that dark narrow bands of much branched irregular mycelium are produced, while wide light colored bands are produced from growth at high temperatures. Growth was good at 24° C. with no traces of zonation but was so slight at 12° C. that it could scarcely be measured.

Table X

The effects of temperature on zonation in strains of C. beticola

Strain	Corn meal agar			Oat meal agar		
	24° C. Constant	24-12° C. Alternating	12° C. Constant	24° C. Constant	24-12° C. Alternating	12° C. Constant
C-1	0	+ distinct	0	0	+ distinct	0
C-2	0	+ trace	0	0	do	0
C-3	0	+ distinct	0	0	do	0
C-4	0	do	0	0	----	0
C-5	0	do	0	0	+ distinct	0
C-6	0	do	0	0	do	0
C-7	0	+ very faint	0	0	+ trace	0
C-8	0	+ distinct	0	0	do	0
C-9	0	do	0	0	+ distinct	0
C-10	0	do	0	0	do	0
C-11	0	do	0	0	do	0
C-12	0	----	0	0	+ trace	0
C-17	0	+ distinct	0	0	+ distinct	0
C-18	0	+ trace	0	0	do	0
C-19	0	do	0	0	+ trace	0
C-25	0	----	0	0	do	0
C-28	0	+ trace	0	0	+ distinct	0
C-34	0	+ distinct	0	0	do	0
C-36	0	do	0	0	do	0
C-37	0	do	0	0	do	0
C-38	0	do	0	0	do	0

A set of plates from the 24° C. chamber were kept under a bell jar in the laboratory for two weeks after this experiment was completed. They were exposed to daily fluctuations of light and temperature, but concentric rings did not form in mycelium that was formed under constant temperature and darkness. Zonation did occur from the margin outward in all growth formed under fluctuating conditions. This would seem to eliminate the possibility that zonation in cultures of Cercospora beticola is due to staling products or aging of the culture.

According to Bisby (1), Milburn has submitted evidence to show that medium and not daily fluctuations is the agent involved

in zone formation in artificial cultures of Hypocrea rufa. Cercospora beticola growing on different media varies in color, amount and density of mycelium which materially affects the expression of zonation, but from observations made during the light and temperature experiments and on many other cultures, medium has had no pronounced influence on zonation.

Observations were made of zonation in cultures growing on 12 cc., 25 cc. and 50 cc. of agar as outlined in Table I, page 12, to determine the effects of amount and depth of nutrients. At the end of twelve days, zonation was pronounced in all cultures growing on 12 cc. of agar, with only traces appearing in some cultures growing on 25 cc. of agar. Media that produce a heavy felty mycelium did not show zonation at this time. At the end of thirty days zonation was very pronounced in most cultures on 12 cc. of agar, quite distinct on 25 cc. and only faintly visible on 50 cc. of agar. Variation in width of zones was quite marked on the different depths, despite the fact that differences in linear growth were practically negligible. It was universally true that the widest zones were produced on the thin substrata and the narrowest zones on the deepest substrata. Hence, it appears that medium is a contributing factor only in so far as it affects the expression of growth rings and is not a causative factor in itself.

The study of variant forms

The attention of the writer was first called to variant forms in Cercospora beticola by the occasional occurrence of sectors of different colored mycelium in colonies of the organism growing on plates. At first no particular attention was given to the aberrant sectors until it was observed that they seemed more or less permanent in subsequent transfers if inoculum was carefully selected from the outermost regions of growth so as to take only the aberrant mycelium. These variants always appeared as fan-shaped or wedge-shaped sectors and were noted in practically all of the strains under attention. The aberrants differed from the parental form not only in the color of the submerged mycelium but in the rate of growth of this mycelium. The aerial growth differed also in type, amount, color and density. In the early part of the investigation, only those sectors showing marked differences were selected since they lent themselves more readily to the study. Later, sectors were selected from the various strains under observation which differed less in the color variation. The preliminary transfers of these isolations showed them to be more or less constant, and it seems that the writer is here dealing with a similar phenomenon to that observed and studied by Stevens (16) and Christensen (5,6) in artificial cultures of Helminthosporium sativum, and also by Caldis and Coons (3) in several other species of fungi.

The aberrant forms were isolated from the parental form by picking a few filaments of the derived fungus from the border

of the colony at the center of the sector. If care is taken, pure variant material can be picked out in this way in practically all cases. The variant was then grown on the same plate with the parental form for a number of times in order to test the purity of the material. Out of twenty-odd such selections, in only one case does it seem likely that a portion of the normal mycelium was transferred along with the variant and in this particular instance the first planting gave evidence of the mixture. In a few instances spores were produced on the variant forms, in which case single spore isolations were secured. These cases are indicated in the charts. Morphological studies were made of the variant of strain C-1. Study of the other forms was limited largely to the physiological activities.

The following system was used in keeping the records of the various cultures used in this work. The various strains of Cercospora beticola used in this work were labeled C-1, C-2, C-3, etc. Variant forms were labeled v/1, v/2, etc., to indicate the number of times the material had been transferred. The original parental material was always grown in parallel cultures on the same plate and labeled as 0/1, 0/2, etc. Thus (C-1 v/7) refers to the variant form of strain one of Cercospora beticola in its seventh transfer and (C-1 0/7) refers to the same strain of Cercospora beticola but is the normal form which has been grown in parallel culture with the aberrant form.

Variants obtained from Cercospora beticola strain C-1

On October 31, 1926, single spored isolations C-1, C-2 and C-3 were made from diseased material collected at Rocky Ford, Colorado. These cultures apparently alike were carried through several transfers as stock cultures, until January 11, 1927 when ten day cultures on beet leaf agar in petri dishes showed achromatic fan-shaped sectors in strains C-1 and C-3. Conidia were formed in the normal green mucelium but more were found on the colorless forms, so on February 15, 1927, mycelial transfers were made from the aberrant sectors as (1 v/7) and (3 v/1) to corn meal agar. The variant form C-3 apparently reverted en masse to the normal form but the aberrant form (1 v/1) remained white with very sparse aerial mycelium. The variant (3 v/1) was discarded, being considered not permanent, but the variant form of strain C-1 was transferred to corn meal (1 v/2) in a parallel culture with the original form (1 0/2). On March 14, 1927, transfers from the pair (1 0/3) and (1 v/3) were made to corn meal agar in test tubes. No further work was done with these forms until October 3, 1927, when transfers (1 0/4) and (1 v/4) were made to corn meal agar plates. During this period of almost six months the cultures had become dried and it was necessary to pour nutrient broth into the test tubes. The growth thus obtained was typically green and achromatic.

The most obvious difference between the normal form and its derivative obtained from strain C-1 was the color of the

colony and the amount and density of the aerial growth (Plate IX, Fig. 1). These differences were so marked that the variant could easily be mistaken for a contamination rather than Cercospora beticola. However, physiological and morphological studies have proved beyond a doubt that the aberrant form is Cercospora beticola. Moreover, these studies have brought out certain aberrant characteristics not discernible under ordinary observation.

Physiological activities Parallel cultures of the normal and the aberrant forms have been grown on many media under a wide range of environmental conditions (Plate III, Fig. 1). On artificial media the physiological characteristics have been constant throughout twenty transfers over a period of sixteen months. Table XI shows the rate of linear growth and other cultural characteristics of the two forms growing on twelve different media. The data were taken after ten days growth. The contrast in linear growth was quite marked, varying somewhat with the medium, but generally being more rapid in the variant form. Amount and density of the aerial mycelium also varied. As will be seen in the table, it was abundant but appressed and felty and on other media almost lacking. With continued cultivation there has been a gradual decrease in the amount of aerial mycelium produced by the variant form on corn meal agar until at present it is entirely absent except for a tuft in the center. Passage through the host plant has had a general tendency to restore the color and amount of aerial growth, isolations

Table XI

Growth of C. beticola variant form (C-1 v/O) and its parental form C-1

Agar medium	Parental			Variant		
	Diam. m/m	Submerged	Aerial	Diam. m/m	Submerged	Aerial
Plain	23	Pale green	Slight, gray	27	Colorless	None
Prune juice	24	Olive green	Abundant, gray, felty	36	do	Sparse, tufted
Litmus lac.	19	Dark green	Low, felty, grayish-green	24	do	Low, wet, felty
Beet leaf	19	Greenish-black	Short, dark, mealy conidia	22	do	None
Malt ext.	24	Greenish-gray	Abundant, felty, gray	28	do	Abundant, low
Neut. red	9	Black	Tufted, grayish-black	13	do	Tufted
Endo's	16	Green	Short, deep greenish-gray	20	do	Growth sparse
Lima bean	24	Greenish-black	Abundant, light gray, felty	23	do	Sparse, wet, felty
Bean pod	25	Greenish-black	Abundant, gray-green, felty	27	do	Sparse
Oat meal	21	Light green	Sparse, greenish-gray	24	do	Sparse
Pot. dex.	12	Olive green	Abundant, greenish-gray	20	do	Sparse
Corn meal	25	Olive green	Greenish-gray	28	do	Sparse

from plants being pale green with considerable aerial mycelium.

Pathogenicity Infection of plants by this variant has been very difficult to secure, evidently due to its low virulence. Inoculations from beet leaf agar cultures have been tried repeatedly under the most favorable conditions for infection. A few scattering spots have been formed but conidia were not produced, the spots remaining as small brown flecks.

Morphological characters Camera lucida drawings of the submerged mycelium from cultures on various media are shown in Plate II, Fig. 1,2. The mycelium of the aberrant form is characterized by being smaller and less tortuous, and more hyaline with fine granular cyloptasm, with fewer of the large translucent oil globules indicating a different food storage capacity than the parental form. The loose sclerotium-like bodies so often found in the submerged mycelium of the normal form have never been observed in this aberrant form.

As previously stated, the length of conidia and the number of septae per conidium in Cercospora beticola are variable factors depending somewhat upon nutrients, humidity and temperature. The variant form of strain C-1 has been practically sterile throughout its cultural history but a few very small conidia were found produced on (C-1 v/7) growing on beet leaf agar. Measurements were made of twenty conidia picked at random from washings of the culture and compared with a like number from (C-1 0/7), the homologous culture of the original material.

Table XII

A comparison of conidia produced by the variant (C-1 v/7) and its parental form (C-1 0/7)

Form	No. of septae			Length in m/m			Width in m/m
	Max.	Aver.	Min.	Max.	Aver.	Min.	
C-1 0/7	22	10.8	4	360	138	55	4-5
C-1 v/7	6	4.6	2	52	35	24	3.2-4.3

The data given indicate that the differences in average size of conidia and the number of septae per conidium between the parent and variant forms are very great. The spores produced by the variant form are more hyaline with more finely granulated cytoplasm than those from the normal form. No difference could be noticed in germination of the conidia in hanging drops of distilled water and nutrient broth. Subcultures arising from single conidia obtained from the variant culture gave colonies indistinguishable from their source.

Further occurrence of variant forms in strain C-1 On December 2, 1927, an achromatic sector was observed in a petri dish culture of strain C-1 whichup to this time had been carried in stock culture. A mycelial isolation was made from the variant sector which was recorded as (C-1 A v/0). On January 5, 1928, culture (C-1 0/6) growing on prune juice agar gave rise to an aberrant sector (C-1 B v/0) which was very similar to (C-1 A v/0). These two variants resembled (C-1 v/0) as originally isolated and so these new variants were not studied in such detail but were transferred just often enough to maintain

new growth at all times. They are now in the sixth and fifth transfers respectively and so far have shown no tendency to revert to the normal form. As in (C-1 v/0) there has been a steady decrease in the amount of aerial mycelium and from all appearances these derivatives of strain C-1 seem identical.

Variants obtained from Cercospora beticola strain C-9

Diseased material showing heavy infection of Cercospora leaf spot was received from Grand Island, Nebraska, on September 2, 1927, from which single spored cultural strains C-9, C-12 and C-14 were obtained by the dilution plate method. Variant forms have arisen from strain C-9 at two different times, but were not recognized as like forms until cultures growing on corn meal agar were compared. After this similarity was observed, comparisons were made on several media and the similarity of behavior has been constantly noted.

Aberrant form of Cercospora beticola strain C-9 (C-9 v/0)

This aberrant form as outlined in the chart (Plate IV, Fig.3) originated as a fan-shaped sector in strain C-9 growing on beet leaf agar. The sector was white with sparse, sterile aerial mycelium. Sub-cultures of the aberrant and parental forms (9 v/1) and (9 0/1) were made on corn meal agar. Growth in the substratum was light green with fine mycelium. Zonation was very pronounced under exposure to ordinary daylight. Aerial mycelium was almost absent in the younger growth but appeared as a white tuft in the center of the 10 day old cultures, becoming

grayish-white in older cultures. These aberrant cultural characteristics are in contrast with the heavy olive green submerged mycelium and the gray-green aerial growth of the parental form. Further, the original form also produced a reddish tinge in the medium, and had abundant aerial mycelium which in older cultures had a tendency to clump. This variant form has been carried on artificial media in parallel transfers with its parental form (C-9 O/O) through fifteen successive transfers over a period of six months. A sector of the variant form in transfer (2) reverted to the normal form as is shown in the chart. The aberrant and its parental form were grown on beet leaf agar in transfer (8) for the first time since the occurrence of the variant form. The aberrant characteristics were still present, growth being almost colorless, zonation very pronounced and the aerial mycelium sparse and practically sterile.

Passage through plants has twice been successful without any marked change in pathogenicity. The slight difference in virulence of the two forms can probably be explained by the absence of conidia in the inoculum of the aberrant form (C-9 v/8). Conidial production in spots produced by the aberrant form took place as in the parental culture, and the spots when old became typically ashen-gray from the conidiophores and conidia that were produced.

Isolations of single spores were made from these inoculated plants to see what effect passage through the host plant had on

the cultural characteristics of the aberrant form. The color of the submerged mycelium which arose from such isolations was practically restored to the normal olive green of the parental form. It was somewhat lighter in color than the parental form (C-9 0/0) after passage through plants, but had lost the pale yellowish green of the variant form (C-9 v/13) which had been grown continuously on artificial media. Other aberrant characteristics were apparently unaltered. Zonation was more pronounced and no sclerotium-like bodies were observed in the submerged mycelium of the re-isolated aberrant form. Aerial mycelium was not produced in these cultures until about the tenth day and then only as a white tuft in the older growth, a thing which was very characteristic also of the aberrant form (C-9 v/13).

Additional aberrant forms of Cercospora beticola (C-9 A v/0) - (C-9 B v/0) These variant forms originated in cultures of strain C-9 growing on beet leaf agar after the organism had been passed through the host plant as is shown in the chart (Plate V, Fig. 1). As previously stated, these isolations resemble the variant form (C-9 v/0) very closely and have been considered identical with the first variant obtained from this strain of Cercospora beticola. As the chart shows, they have received essentially the same treatment as the first variant isolated (C-9 v/0) since their isolation and have behaved very similarly so a detailed discussion will not be given. Passage

through plants failed to reveal any marked difference in pathogenicity and produced comparable results with those obtained in the first variant form (C-9 v/0).

The salient features of the work with the variants from this form seem to be, (1) the same sort of variant has been thrown three times by this culture, (2) the depth of color which is a more or less characteristic thing for the variant varies with the medium and passage through plants tends to restore this color, and (3) the mycelial characters of the variant are more or less constant, apparently being unaltered by passage through the natural host plant.

Variants obtained from Cercospora beticola strain C-8

Cultures C-7 and C-8 were obtained by single spore isolations from diseased material received from Chaska, Minnesota, on September 1, 1927. They were typical Cercospora cultures with olive green mycelium in the substrata and gray-green aerial mycelium. Culture C-7 has never given rise to aberrant forms but three distinctly different variants have been obtained from form C-8. On January 1, 1928, thirty-nine transfers of C-8 were made on thirteen different media for the purpose of studying the effect of type and amount of nutrients on the cultural characteristics of Cercospora beticola. Cultural variations were pronounced but all transfers except one apparently were homogeneous. This transfer on 35 cc. of litmus lactose agar in a petri dish produced an aberrant sector, which at ten days

was darker colored, with low, compact aerial growth (Plate VII; Fig. 3). Mycelial transfers from the aberrant sector to corn mean agar (C-8 v/1) produced a colony deep olive green in color, with dense deep greenish-gray aerial mycelium which in older cultures develops a pronounced tendency to clump into loose masses. The normal form C-8 0/1) on corn meal agar was slightly lighter colored, producing a wine red tinge in the medium. It has light colored, less abundant aerial mycelium, which shows less tendency to form in clumps than the variant form (C-8 v/1). Cultural washings from (C-8 0/8) and (C-8 v/6) on beet leaf agar indicated that conidia production was heavier in the aberrant form. Inoculation on plants produced heavier infection in the aberrant form, fifteen days after inoculation. Passage through sugar beets did not affect the aberrant characteristics in subsequent transfers. There was a slight tendency toward a deeper color in both the normal and the aberrant forms that had passed through plants over those carried continuously on artificial culture media through thirteen transfers.

Aberrant form (C-8 A v/o) The transfer of strain C-8 on 12 cc. of oat meal agar of January 1, 1928, was abnormally pale green with very sparse aerial mycelium. A transfer was made to corn meal agar to determine the effect of nutrients on color. This culture was normal green with abundant greenish-gray aerial mycelium. On January 30, 1928, a transfer was again made to oat meal agar which developed into a pale green culture having

very little aerial mycelium, with a large achromatic sector with abundant loose aerial mycelium. A transfer from the aberrant sector to corn meal agar (C-8 A v/1) produced a pure achromatic culture with a reddish tinge in the substratum. A sector of the culture reverted to the normal as is shown in the chart (Plate IV, Fig. 1) but subsequent cultures from the achromatic portion have remained constant through nine transfers on artificial media. Inoculations of the aberrant form (C-8 A v/7) on sugar beets produced a very light infection. The diseased areas were typical, producing a few normal conidia. Isolations from these plants produced only normal green cultures comparable with those isolated from plants inoculated with culture (C-8 A 0/7). In this form we have aberrant characteristics that were constant on artificial media but were not constant through plants indicating that the variation was in some way connected with malnutrition of the fungus.

Aberrant form (C-8 B v/0) The plate transfer of the parental form (C-8 A 0/3) gave rise to an aberrant sector which had yellow mycelium. Aberrant form (C-8 A v/0) was white, hence this variant appeared to be distinct from it. This variant has remained constant through five successive transfers on corn meal agar.

Variants obtained from Cercospora beticola strains C-19 and C-20

Diseased material was received from Grand Island, Nebraska, on September 4, 1927, from which single spore culture strains C-18, C-19 and C-20 were obtained by the dilution plate method. These cultures were carried in stock cultures without producing aberrant forms until January 1, 1928, when variant sectors appeared in plate cultures of strains C-19 and C-20 growing on corn meal agar.

Variant obtained from Cercospora beticola strain C-19 (C-19 v/0) The variant sector in strain C-19 was yellow, producing a red pigment in the substratum, with sparse light colored aerial mycelium, while the rest of the colony was normal olive green in the substratum with greenish-gray aerial mycelium. When sub-cultured from mycelium to corn meal agar the aberrant form became a pale yellowish green producing a pronounced wine red tinge in the substratum, with grayish-white aerial mycelium in a tuft at the center of the colony. The parallel transfers of the normal form (C-19 0/1) was normal in all respects. These characters were constant throughout the eleven transfers on corn meal agar. On prune juice agar the aerial mycelium in both the parental and the aberrant forms was lighter in color and more felty. When grown on beet leaf agar the aberrant form becomes almost achromatic in the substratum with pure white aerial mycelium. No appreciable difference in pathogenicity of the two forms for sugar beet could be noticed through

artificial inoculations. Conidia were produced in the typical small brown diseased areas on the plants inoculated with both the normal and the variant forms. Cultures isolated from the plants inoculated with the variant form were somewhat darker in color than the line carried continuously on artificial media. The color of the submerged mycelium was somewhat lighter green than the color of the normal form and the wine red pigment of the aberrant form was much more pronounced in those isolated cultures than it was in the form kept constantly under artificial culture. The aerial mycelium was changed from a grayish-white to a dark gray but no increase in density was noted in the organism passed through plants.

Aberrant form (C-19 A v/0) On February 1, 1928, a transfer of C-19 was made from the stock culture of December 30, 1927 for comparison with (C-19 0/3). An aberrant sector identical with (C-19 v/0) was produced in this transfer, so on February 10, 1928, a mycelial sub-culture was made from this sector and recorded as (C-19 A v/1). Since the time of isolation this aberrant form has received the same treatment as the variant (C-19 v/0) and has been found to be identical with it in every respect, so much so in fact that they cannot be distinguished from each other.

In these two variant forms we have sudden variations occurring whose stability at least in part depends somewhat upon the medium. The normal color is at least partially

restored when the variants are cultured on the natural host plant. It is interesting to note that the color of the parental form was somewhat intensified by passage through sugar beets. The other aberrant characters were apparently unaltered by passage of the variant forms through the natural medium.

Aberrant form (C-30 v/0) This form originated as an aberrant sector in strain C-30 as stated above. The growth in the substratum was a pale yellowish green in contrast with the olive green of the normal portion of the culture. The aerial mycelium was abundant, loose and uniformly yellow, as contrasted with the greenish-gray, rather sparse aerial growth of the parental form. There has been a gradual decrease in color of the submerged mycelium until in the eleventh transfer of the variant form (C-30 v/11) it is almost the color of the aerial mycelium. When grown on beet leaf agar the aberrant form becomes almost achromatic with abundant felty almost sterile aerial mycelium. The aberrant form seemed to produce a lighter infection on sugar beets than did the parental form. The symptoms were typical, the diseased areas becoming ashen gray with conidia. Isolation of the variant form from inoculated sugar beets showed that passage through plants had restored the normal olive green color but did not affect the type of aerial mycelium, which was more abundant and felty that it was in parallel cultures of the normal form (C-30 0/0). The color had been changed from yellow to a

grayish yellow. Transfer (3) of the aberrant form produced a sector which was normal in every respect, through two successive transfers on artificial media.

Variant obtained from Cercospora beticola strain C-17

Culture strains C-15, C-16 and C-17 were isolated by the dilution plate method from diseased material received from Mason City, Iowa, on September 4, 1927. Approximately one-half of a transfer of C-17 growing on a beet leaf agar plate of January 3, 1927 was achromatic with sterile white aerial mycelium, and the rest of the colony was normal dark green with dark colored aerial growth. When grown on corn meal agar the variant became pale yellowish green in the substratum with sparse light colored aerial mycelium. Zonation was faint in the aberrant form and very pronounced in the parental form. Sub-cultures to beet leaf agar as indicated in the chart (Plate V, Fig. 2) were somewhat lighter in color than the original form (C-17 v/O) but were not pure achromatic as was the aberrant sector. There was no appreciable difference in pathogenicity between the two forms when inoculated on sugar beets. Passage through plants had a tendency to intensify the colors as described in the discussion of variant form (C-9 v/O) but did not eliminate the aberrant characteristics. These variant characters are somewhat variable on media but have been constant throughout the culture on artificial media.

Attempts to produce variants

As detailed by Brierley (3), various workers have produced "mutations" in Aspergillus niger and Penicillium glaucum by treating the cultures with various chemicals. Brierley (3) has tried to repeat this work, using strains of the same fungi, without success. Stevens (16) reports unsuccessful attempts to produce "saltations" in Helminthosporium sativum by wounding and various other methods. The writer has carried out the following outlined attempts to produce aberrant forms in normal cultures of Cercospora beticola.

Wounding The tips of actively growing hyphae in cultures C-1 and C-27 growing on corn meal agar were killed by means of a hot platinum wire in several places around the border of the colony. The injured areas soon outgrew the injury, the margins of the colonies presenting a uniform appearance. These results are strengthened by the fact that variations have never been found to arise in cultures at the point from which inoculum has been procured with a hot needle.

Illuminating gas Since gas poisoning of cultures is always a possibility under laboratory conditions, the writer sought to determine if this factor was responsible for sector formation in normal cultures. Two week old cultures C-1 and C-27 were placed in a series of air-tight chambers and treated with illuminating gas for 4, 6, 8, 10 and 24 hour periods. The gas was allowed to pass through the chamber for thirty

minutes, thus forcing out all of the air. Cultures exposed to 4 and 6 hour treatments were only slightly inhibited in growth as compared to the checks. Eight and ten hour treatments inhibited growth completely but did not kill the organism as inoculum put on corn meal agar produced normal growth, while twenty-four hour treatment killed the fungus. The cultures were kept in the laboratory for three weeks after treatment without any sign of variation, nor have any of the subsequent transfers given rise to aberrant forms.

Freezing Since many of the aberrant forms under observation have appeared during the winter months, cultures of the organism were frozen with carbon dioxide gas in an attempt to induce formation of variant sectors by exposure to extremely low temperatures. The methods of freezing were varied, one to thirty minutes continuous freezing being applied to some cultures while others were alternately frozen and thawed for different lengths of time. The treated cultures were allowed to grow for ten days and then sub-cultures were made from the frozen areas, but none of the treated cultures or the subsequent transfers gave any indications of throwing variants.

Poisoning with chemicals Vigorously growing cultures of the fungus were treated with chemicals chosen because they were strong oxidizing or reducing agents in an attempt to induce the throwing of variants. Small crystals of $K_2Cr_2O_7$ and drops of solutions of KNO_3, H_2O_2 and CHOH were placed at

different points around the margins of the colonies. In this manner different concentrations were obtained as diffusion of the chemicals through the medium took place outward from the points of application. The fungus was killed at the points of application, and growth was inhibited to a variable degree depending upon the concentration of the chemicals, but subsequent growth was normal, with no indication of variants. Sub-cultures made from different portions of the treated colonies gave only normal forms.

These attempts and many others of a similar nature have been tried at various times by the writer but always with negative results. They indicate that the coarse methods used were not so directed as to upset the equilibrium within the fungus cells, rather than exhausting the possibilities of success through methods of this sort.

Mechanics of sector formation

Artificial sectors were produced both by mixed planting and implanting of two forms in an attempt to show the mechanism of sector formation. Stevens (16) used these methods in an attempt to demonstrate that ordinary transfers might be mixtures of two forms or races, thus explaining the appearance of "saltations". He obtained no segregation of forms in mixed plantings and only partial success by implantings.

Implantings of mycelium from an achromatic form at the border of a normal green culture gave rise to typical sectors

only when the implanted material had a chance to start active growth before the advancing mycelium of the normal form engulfed it, in which case the planting was obscured. If the implanted material starts growth a typical wedge-shaped sector is formed. There seems to be very little repulsive influence between forms of Cercospora beticola as two forms will grow parallel with each other but with very little intermingling of hyphae. The forms can be isolated in pure culture from the borders of the colony.

Mixed plantings Acting upon the knowledge that the variants were frequently more rapid growers than the parental form, mixed plantings were made to determine whether or not the two forms would segregate and remain distinct. Equal amounts (one square centimeter) of agar and its contained mycelium from each form were thoroughly mixed under sterile conditions, and the mixture used as inoculum on corn meal agar plates. Several forms have been treated in this manner but strain C-1 and its variant, and strain C-8 and its variant have shown the best contrast. Segregation takes place in the form of several wedge-shaped sectors resembling the normal and the aberrant sector from which the forms were obtained (Plate VIII, Fig. 1,2).

The results of this experiment seem very important in explaining what happens in the case of naturally developing sectors. If in a culture two elements of different potentialities develop, then if the growth rates are approximately the

same or if one is slightly greater than the other, there will be produced sectors of the one form in the other. The results from mixing the mycelium together would indicate that a small amount is sufficient to inaugurate a sector; perhaps only a single cell would be sufficient if not repressed by the surrounding growth.

Apparent reversions

Several instances of apparent reversion of the aberrant characteristics have been observed. Such reversions always occurred as normal sectors in the aberrant form, when the variant characters were colony color, amount and density of the aerial mycelium and partial sterility. Achromatic sectors have appeared on beet leaf agar that immediately reverted en masse to the normal form but these variants have not been considered as being stable and only reversions of the stable forms through sectors are here considered.

Reversion has been found to be most common in forms just isolated from the aberrant sector. Aberrant form (C-13 v/0) has shown a great tendency to split up into normal and variant forms. Continued selection for the contrasting types has finally resulted in an achromatic variant (C-13 v/7) that has been stable through the last three transfers. Variant form (C-20 v/3) developed a normal sector in a five day culture that was identical with the parental form in every respect through two subsequent transfers and the variant form has shown no further

tendency to split into the two forms. Variant forms (C-6 v/1), (C-14 v/3) and C-37 v/1) all showed tendencies toward reverting to the parental form but selections for the aberrant characteristics have resulted in phenotypic cultures.

The production in these variant forms of the duplicate of the parental forms, under conditions where it seems that mixture of the parental material was excluded, seems very significant.

Discussion

The development of variations in the mycelium of Cercospora beticola has been noticed throughout the two years of study of the various strains.* These variants have appeared as wedge-shaped sectors in normal cultures, being most noticeable for their lack of color, sparse aerial mycelium and sterility on beet leaf agar. Some of these have reverted more or less to the normal form in color but the mycelial characters have remained constant, intensity of color only being influenced by the medium on which the organism is growing. Some of the aberrant forms (C-1 v/0) in particular have been remarkably permanent on artificial media. Passage through plants has a tendency to intensify the color of the mycelium. Variant forms (C-1 v/0) and (C-8 v/0) were pure white when growing on artificial media but became almost normal green after passage through sugar beets. The density and amount of aerial mycelium and type of growth in the substratum were influenced very

little by passage of the aberrant forms through the host plant.

There has been an extensive literature developed on variations occurring in pure cultures of asexual fungi. Brierley (2) and Stevens (6) have compiled complete bibliographies on the subject from which material has been extensively used. Contradictory views as to the nature of these variant forms have been expressed by various investigators. However, the bulk of the literature merely reports the occurrence of such variations along with certain morphological characteristics and physiological reactions without dealing extensively with the genetic nature of the variations. One group of investigators believe these variant forms to be true "mutations" or "saltations" comparable to the so-called bud mutations which occur in apparently pure line cultures of plants, of which view Stevens (16) and Christensen (5,6) are the strongest exponents. Opposed to this view are those who believe, with Caldis and Coons (3) that they are modified forms of a more or less permanent nature. They have based their belief upon the assumption that if a change in the genetic constitution of the organism (as Brierley (2) has defined a mutation) had actually taken place, the very common occurrence of reversion to the normal form would not then be likely.

Whether or not the variations studied and recorded in this paper are "mutations" or "saltations", or are merely

modified forms, it has been found that they are of only a semi-permanent nature. The actual cause of their development remains as yet unknown, but the changes are of such a type as to suggest nutritional disturbances as playing an important role. The sparse aerial mycelium can also possibly be explained as a result of attenuation of the organism through malnutrition.

In the absence of exact knowledge of the life history of the organism other than the asexual stage, conclusions as to the actual nature of the variant forms cannot be drawn, but at least it can be said that the claim that variants represent "mutations" is at least far from being proven.

Summary

Cercospora beticola is the fungus causing Cercospora leaf spot of sugar beets.

The organism was found to grow well on most standard culture media. Light apparently plays a minor role in its development while temperature affects materially the vegetative growth.

Conidia production in artificial cultures was found to be a response to a nutrient stimulus, light and temperature influences being flexible.

Zonation in artificial cultures is due to the differential growth of mycelium and not to conidia production. It has been induced by fluctuating light and temperature conditions. Other

factors influence but do not initiate zone formation.

Variant forms appearing as wedge-shaped sectors were isolated from known single spore cultures of Cercospora beticola. These aberrant forms have been constant on artificial media. Passage of the aberrant forms through sugar beets has had a tendency to restore the normal color but has not materially affected the morphological characteristics.

Attempts to produce variants by wounding, exposure to illuminating gas and low temperatures and by treating with chemicals have been unsuccessful.

The behavior of these variant forms indicates that they may be looked upon as modified forms with nutritional disturbances playing a role in their development.

Bibliography

1. Bisby, G. R.
1935. Zonation in cultures of Fusarium discolor sulphureum. In Mycologia 17:89-95.

2. Brierley, W. B.
1920. On a form of Botrytis cinera with colorless sclerotia. In Phil. Trans. Roy. Soc. London, Ser. B. 210:83-114.

3. Caldis, P. D. and Coons, G. H.
1926. Achromatic variations in pathogenic fungi. In Mich. Acad. Sc. Vol. VI.

4. Chaudhuri, H.
1923. A study of growth in cultures of Verticillium albo-atrum. B. et. Br. In Ann. Bot. 37:519-539.

5. Christensen, J. J.
1926. Physiological specialization and parasitism of Helminthosporium sativum. In Minn. Exp. Sta. Tech. Bul. 37.

6. Christensen, J. J.
1925. Physiologic specialization and mutation in Helminthosporium sativum. In Phytopath. 15:785-795.

7. Coons, George H.
1916. Factors involved in the growth and pycnidium formation of Plenodomus fuscomaculans. In Rept. Journal Agri. Res. V. 5, No. 16.

8. Coons, George H.
1926. Beets of primitive type in disease control. In Yearbook of Agri. 1926.

9. Duggar, B. M.
1898. Leaf spot of the beet. In Cornell Agr. Exp. Sta. Bul. 163.

10. Hedgecock, G. G.
1906. Zonation in artificial cultures of Cephalothecium and other fungi. In Rept. Mo. Bot. Gard. 17:115-117.

11. Klotz, L. J.
1923. A study of the early blight fungus Cercospora apii. Fres. In Mich. Agri. Exp. Sta. Tech. Bul. No. 63.

12. Leonian, L. H.
1924. A study of factors promoting pycnidium formation in some Sphaeropsidales. In Am. Jour. Bot. 11:19-50.

13. Parr, Rosalie
1918. The response of Pilobolus to light. In Ann. Bot. 32:177-205.

14. Pool, Venus W. and McKay, M. B.
1916. Climatic conditions as related to Cercospora beticola. In Journ. Agri. Res. Vol. VI, No. 1.

15. Pool, Venus W. and McKay, M. B.
1916. Relation of stomatal movement to infection by Cercospora beticola. In Journ. Agri. Res. Vol. V, No. 22.

16. Stevens, F. L.
1922. The Helminthosporium foot rot of wheat with observations on the morphology of Helminthosporium and on the occurrence of saltations in the genus. In Bul. Ill. Nat. Hist. Surv. 14:78-184.

17. Stevens, F. L. and Hall, J. A.
1909. Variations of fungi due to environment. In Bot. Gaz. Vol. 48, No. 1.

18. Townsend, C. O.
1915. Field studies of the crown gall of sugar beets. In U.S. Dept. of Agri. Bul. 303.

Supplement

Cultural strains of *Cercospora beticola*

The following is a list of the single spore isolations used in the study of *Cercospora beticola*. The cultures have been termed strains because of the variability in culture characteristics existing between the different isolations. The occurrence of variant sectors is indicated in each strain. Some of these variants have not been studied in detail but all have been isolated in pure culture.

C-1: Isolated October 31, 1926, from diseased material collected at Rocky Ford, Colorado. Aberrant forms (C-1 v/O), (C-1 A v/O) and (C-1 B v/O).

C-2: Duplicate culture of strain C-1. Aberrant form (C-2 v/O).

C-3: Duplicate culture of strain C-1. Aberrant form (C-3 v/O).

C-4: Isolated October 31, 1926, from material collected at Rocky Ford, Colorado.

C-5: Duplicate culture of strain C-4.

C-6: Isolated July 6, 1927 from diseased material received from Oxnard, California. Aberrant form (C-6 v/O).

C-7: Isolated September 1, 1927, from material received from Chaska, Minnesota.

C-8: Duplicate culture of strain C-7. Aberrant forms (C-8 v/O), (C-8 A v/O) and (C-8 B v/O).

C-9: Isolated September 3, 1927 from material received at Grand Island, Nebraska. Aberrant forms (C-9 v/O), (C-9 A v/O) and C-9 B v/O).

C-10: Isolated November 5, 1926 from material collected at East Lansing, Michigan. Aberrant form (C-10 v/O).

C-11: Duplicate culture of strain C-10. Aberrant form (C-11 v/0).

C-12: Duplicate culture of strain C-9.

C-13: Duplicate culture of strain C-10. Aberrant form (C-13 v/0).

C-14: Duplicate culture of strain C-9. Aberrant form (C-14 v/0).

C-15: Isolated September 4, 1927, from material received from Mason City, Iowa.

C-16: Duplicate culture of strain C-15.

C-17: Duplicate culture of strain C-15. Aberrant forms (C-17 v/0) and (C-17 A v/0).

C-18: Isolated September 4, 1927, from diseased material received from Grand Island, Nebraska.

C-19: Duplicate culture of strain C-18. Aberrant forms (C-19 v/0) and (C-19 A v/0).

C-20: Duplicate culture of strain C-18. Aberrant form (C-20 v/0).

C-21 - C-35 inclusive were Michigan State College Experiment Station stock cultures and were not used very extensively so individual strains will not be listed.

C-36: Isolated September 8, 1927, from material collected at Rocky Ford, Colorado. Aberrant form (C-36 v/0).

C-37: Duplicate transfer of strain C-36.

C-38: Duplicate transfer of strain C-36.

Explanation of plates

Plate I. Camera lucida drawings of conidiophores and conidia. Fig. 1, Conidiophores, (a) copy from Duggar, (b) from plants, (c) young conidiophores produced in culture, (d) older conidiophores in culture. Fig. 2, Conidia from different sources, (a) germinating conidia.

Plate II. Camera lucida drawings of the mycelium of strain C-1 and its variant form growing on various media.

Plate III. Fig. 1, Chart showing cultural history of variant form (C-1 v/0). Fig. 2, Chart showing cultural history of variant form (C-8 v/0).

Plate IV. Fig. 1, Chart showing cultural history of variant form (C-8 A v/0). Fig. 2, Chart showing cultural history of variant form (C-9 v/0).

Plate V. Fig. 1, Chart showing cultural history of variant forms (C-9 A v/0) and (C-9 B v/0). Fig. 2, Chart showing cultural history of variant form (C-17 v/0).

Plate VI. Fig. 1, Variant forms (C-19 v/0) and (C-19 A v/0) showing cultural history in chart form. Fig. 2, Chart showing cultural history of variant forms (C-20 v/0).

Plate VII. Fig. 1, (a) differential growth rings in the submerged mycelium induced by alternating temperatures of 25-12° C., (b) Cultures grown at continuous temperature of 25° C. Fig. 2, (a) variant sector in culture of strain C-8 growing on litmus lactose agar, (b) the parental form, (c) the variant form in pure culture.

Plate VIII. Mechanics of sector formation. Fig. 1, Mixed plantings of strain C-1 and its derivative, Fig. 2, Mixed plantings of strain C-8 and its derivative.

Plate IX. Fig. 1, (a) strain C-1, (b) variant from C-1, (c) strain C-8, (d) variant form (C-8 v/0), twenty day cultures. Fig. 2, (a) strain C-1, (b) variant form (C-1 A v/0), (c) strain C-13, (d) variant form (C-13 v/0).

Plate X. Fig. 1, Reversion through sectors in variant form (C-13 v/0). Fig. 2, (a) strain C-8, (b) variant form (C-8 B v/0), (c) strain C-20, (d) variant form (C-20 v/10), all cultures growing on corn meal agar.

Plate I

Fig. 2

Fig. 1

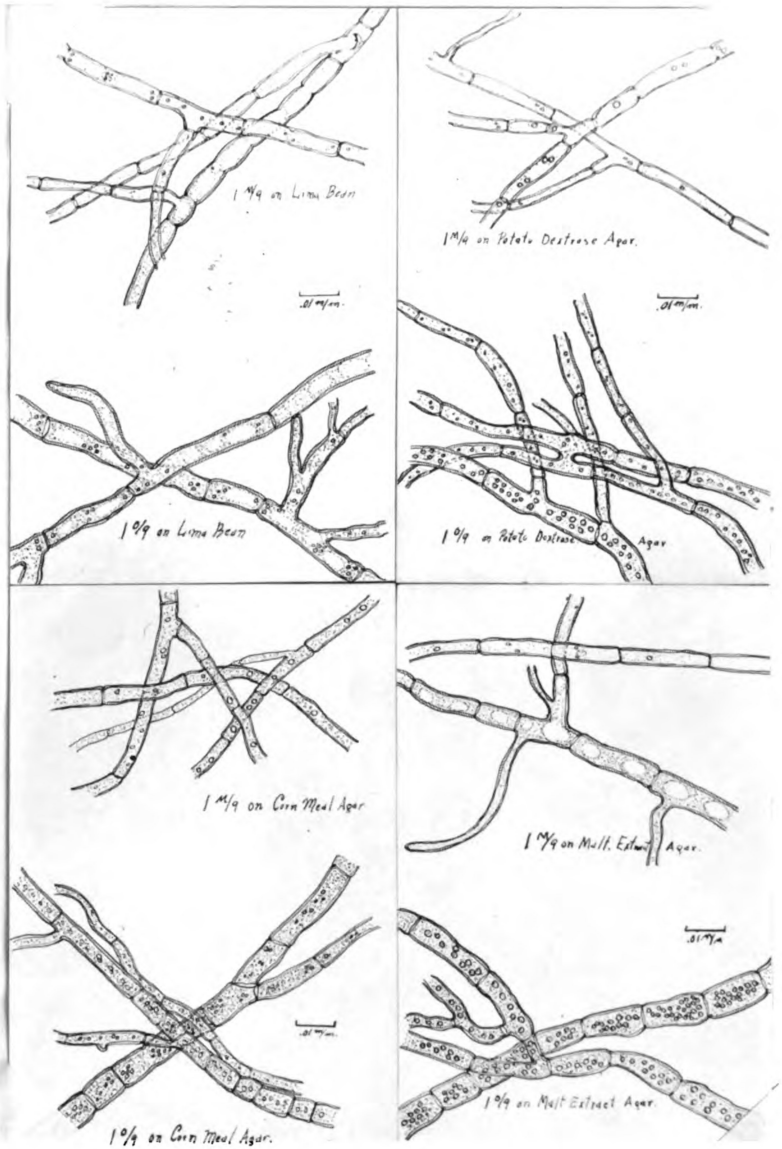


Fig. 1

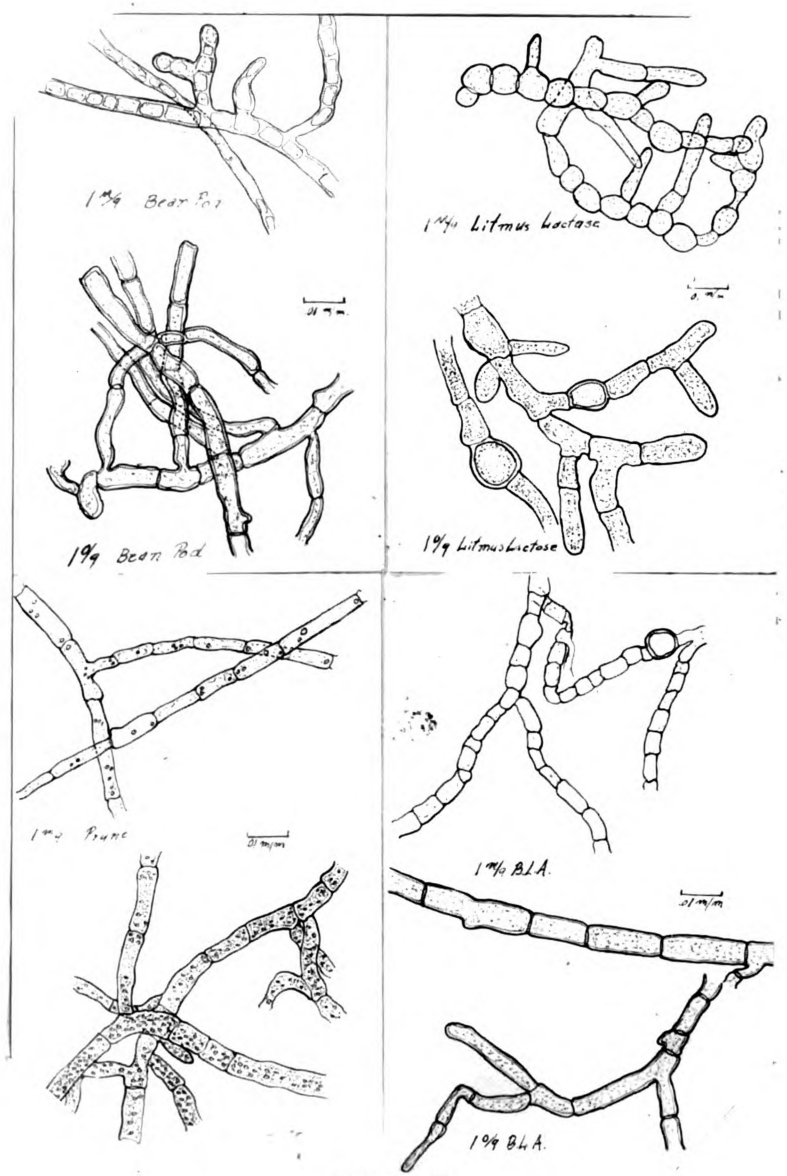


Fig. 2

Plate II

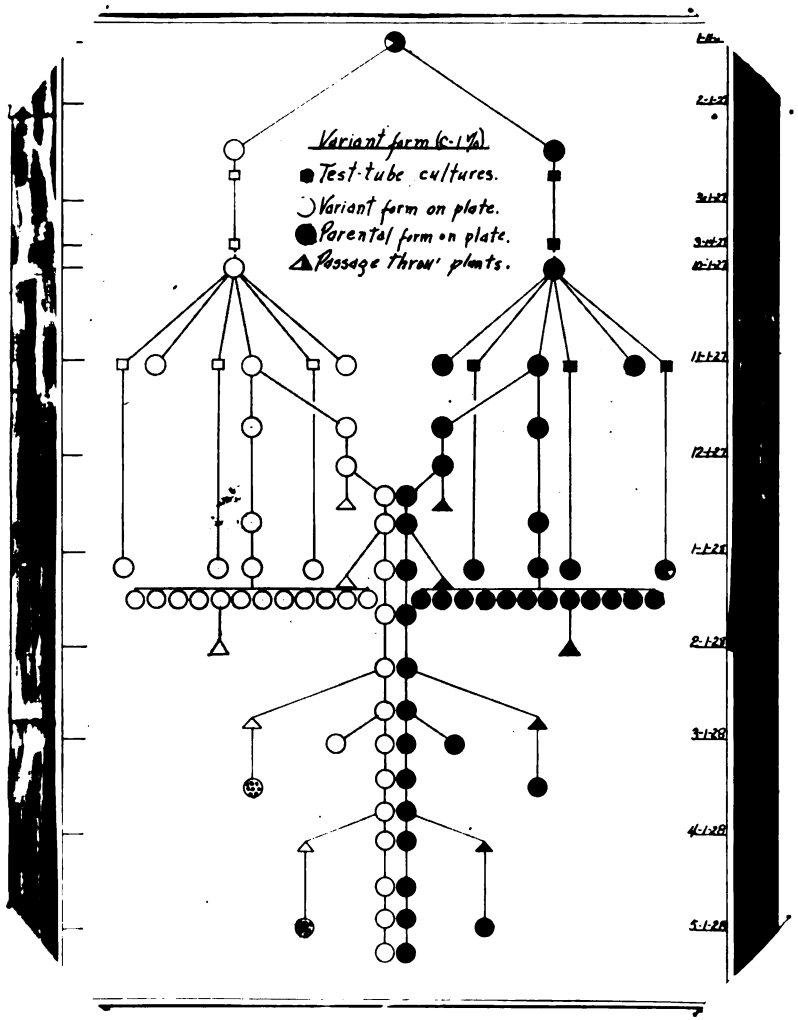


Fig. 1

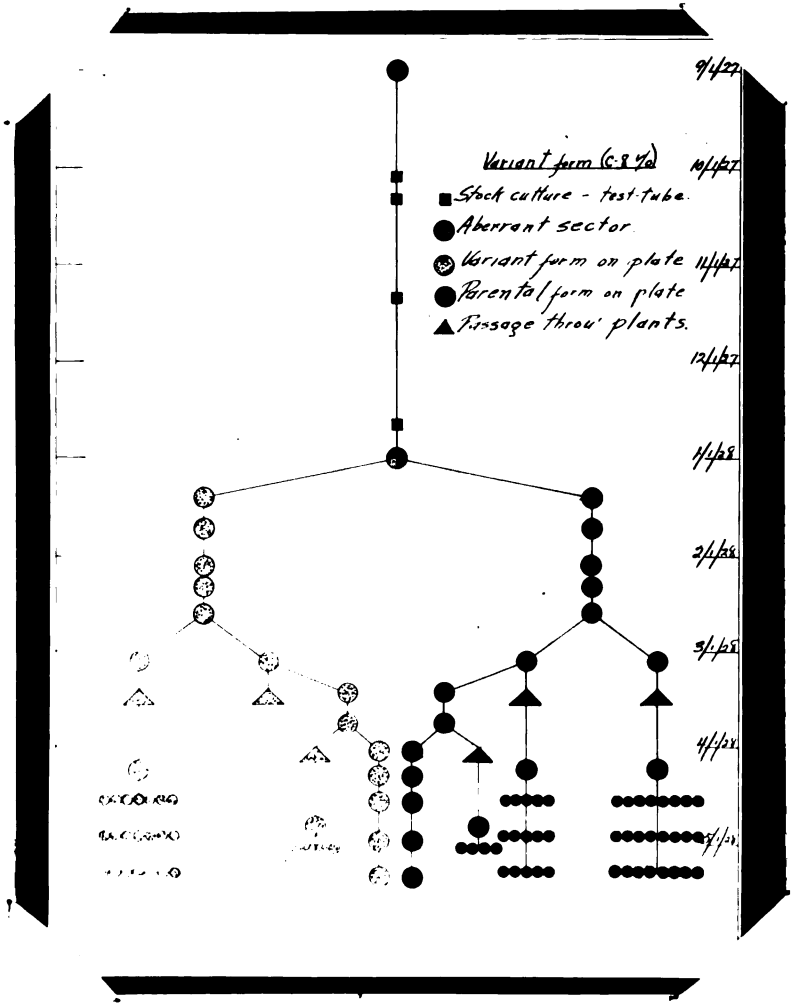


Fig. 2

Plate III

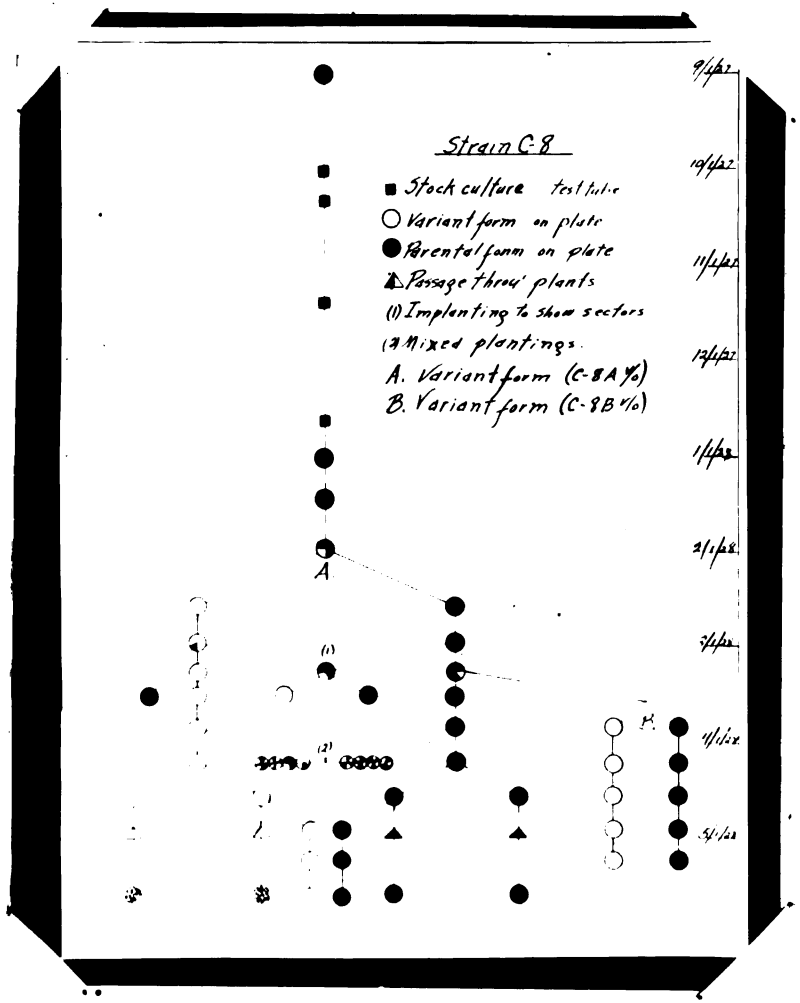


Fig. 1

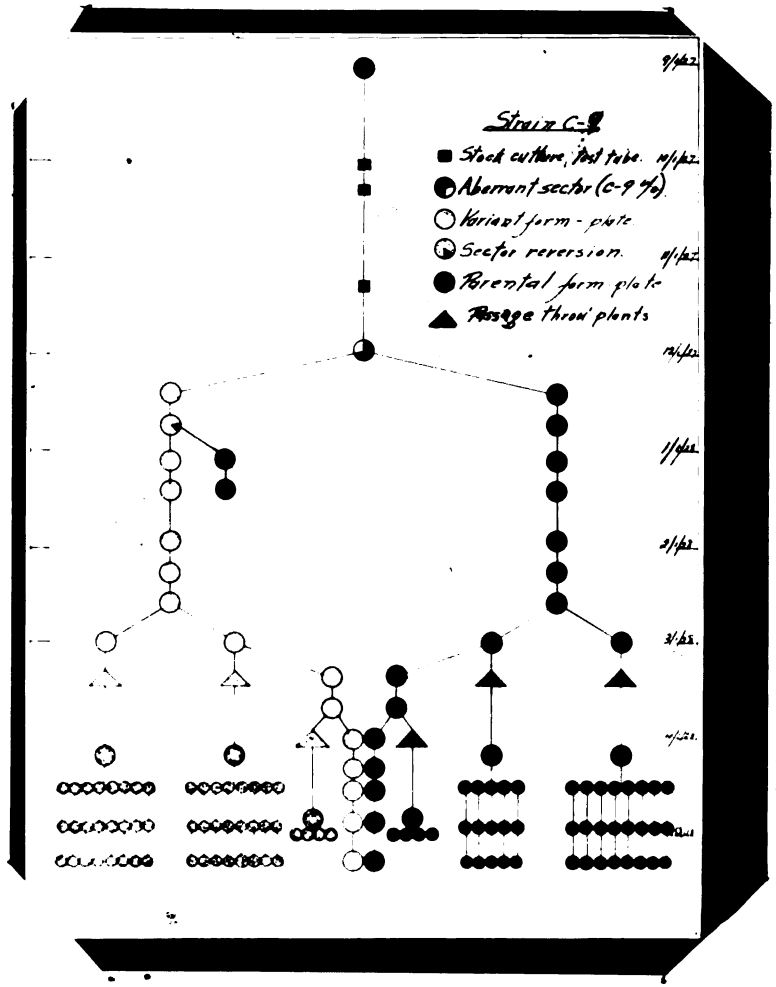


Fig. 2

Plate IV

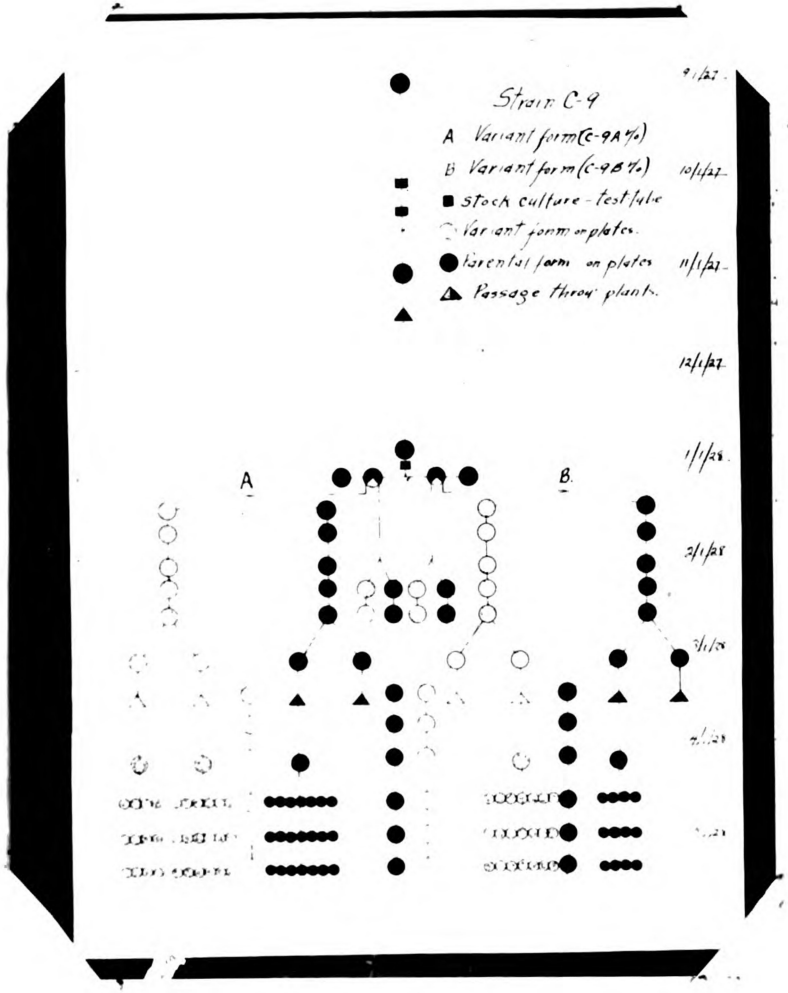


Fig. 1

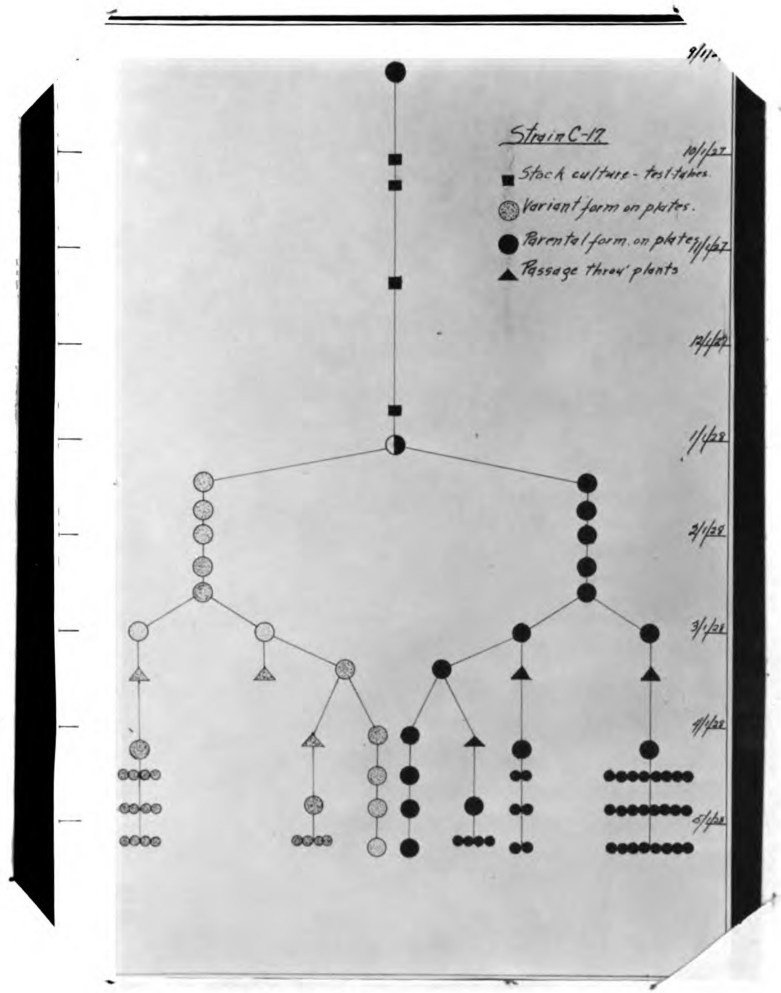


Fig. 2

Plate V

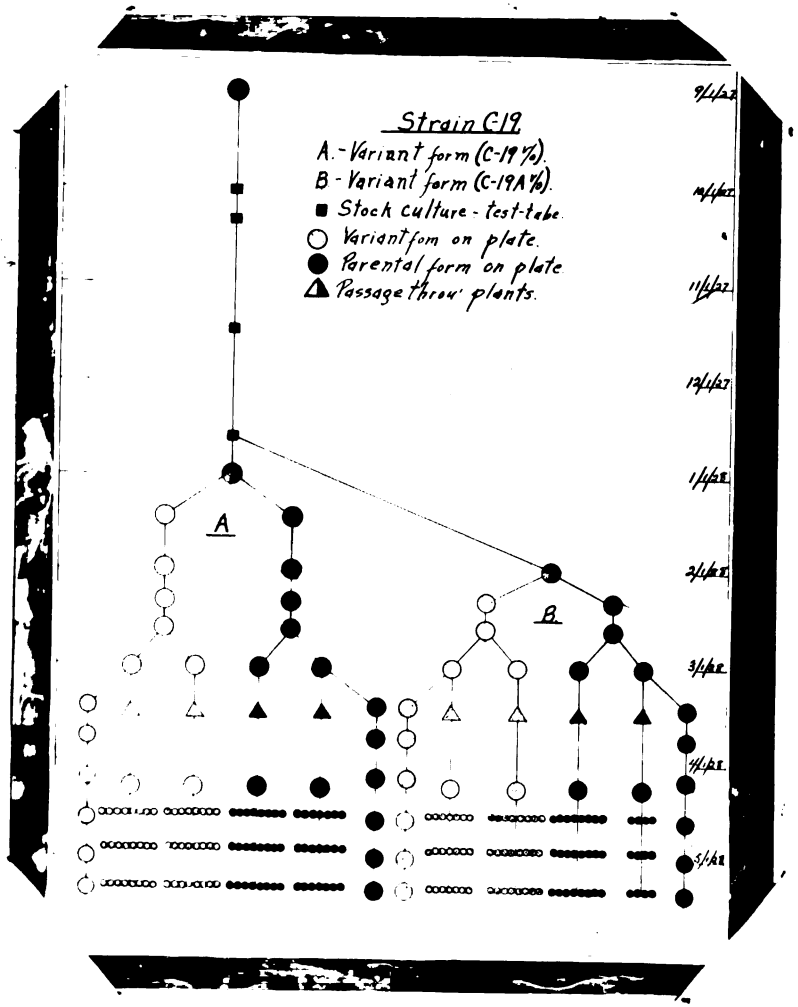


Fig. 1

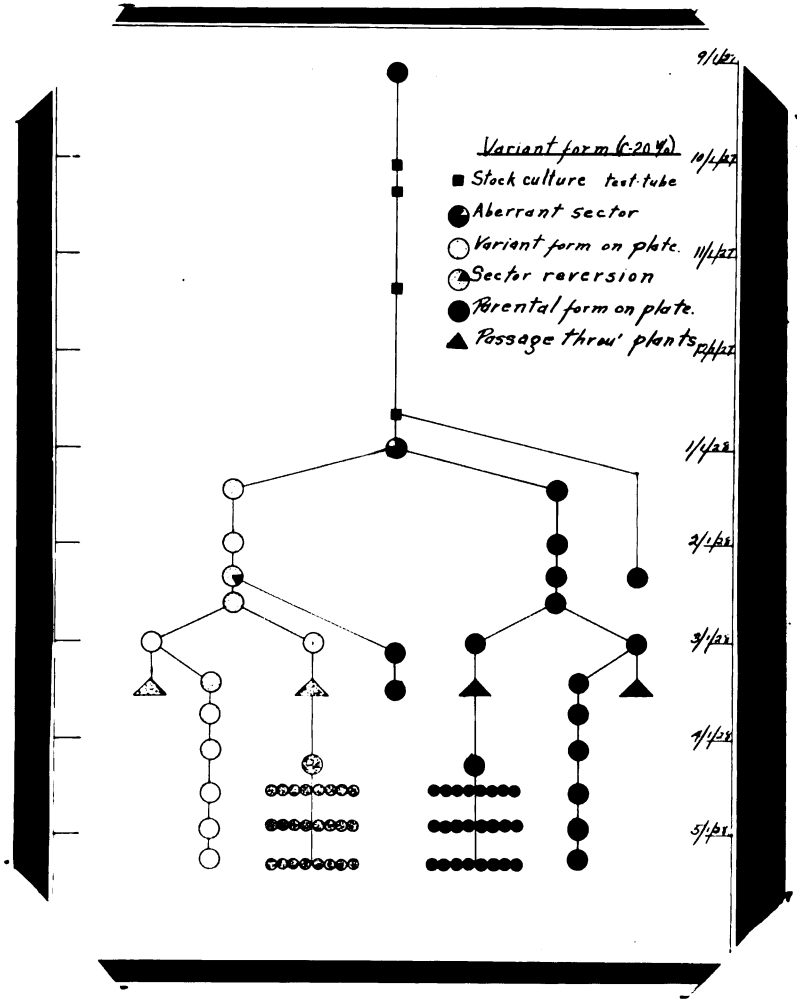


Fig. 2

Plate VI

Plate VII

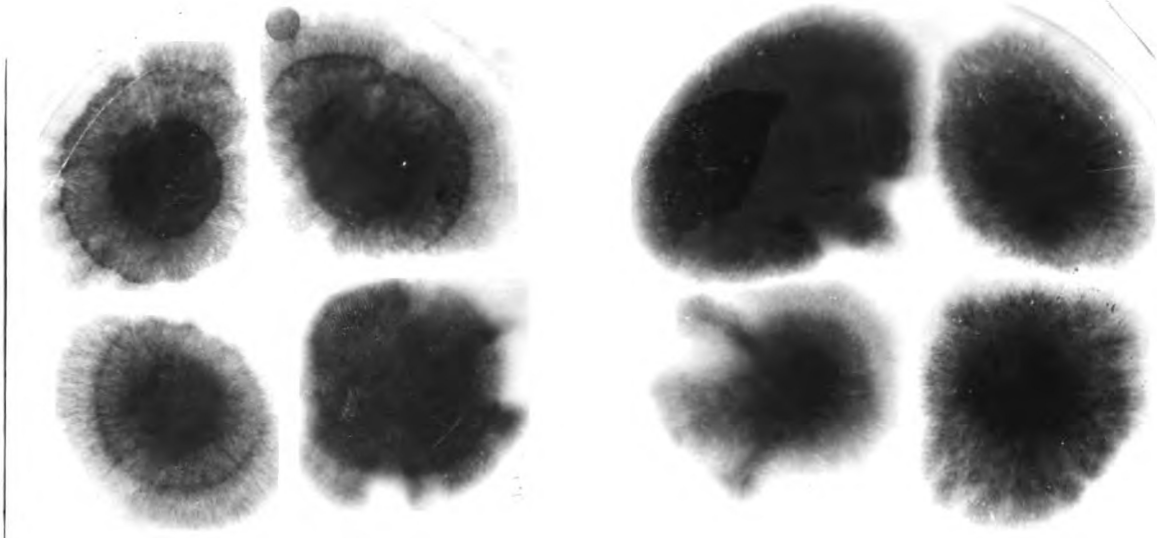


Fig. 1

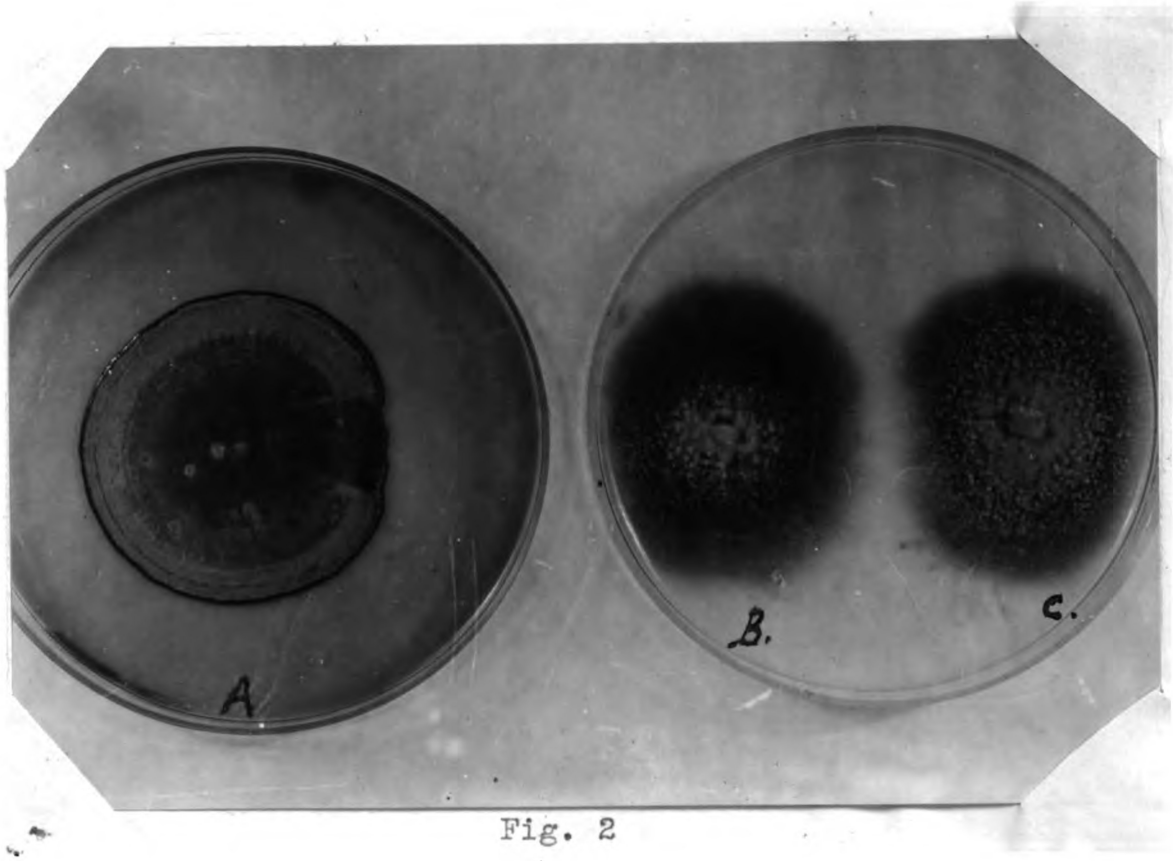


Fig. 2

Plate VIII

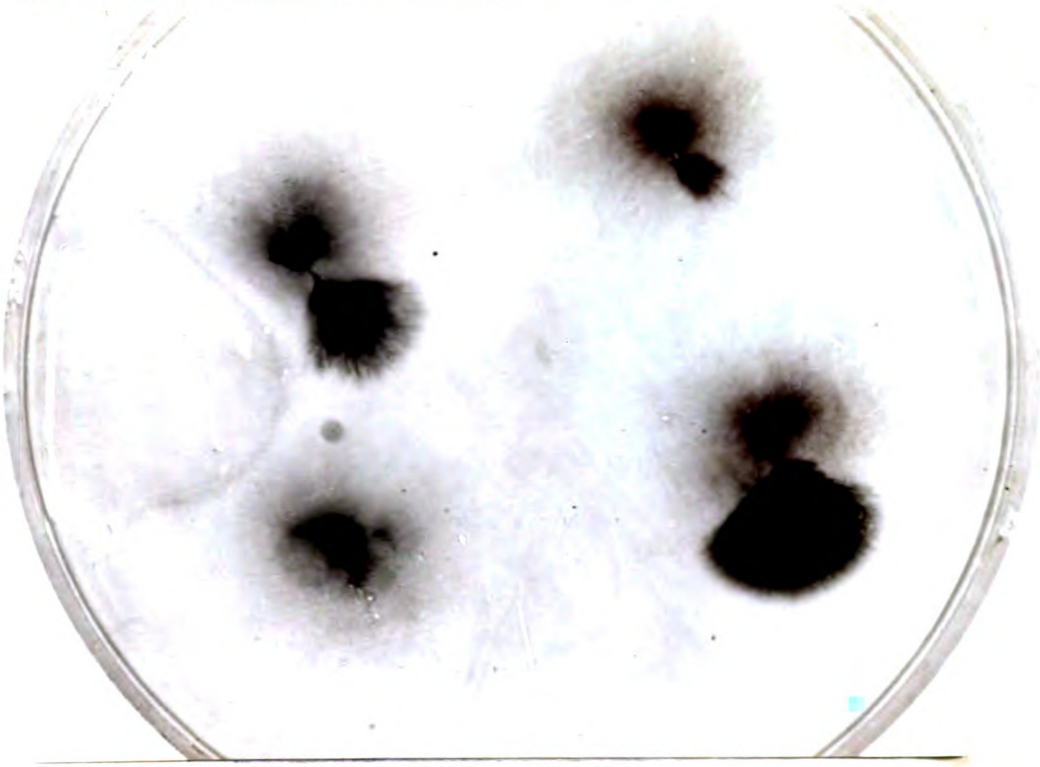


Fig. 1

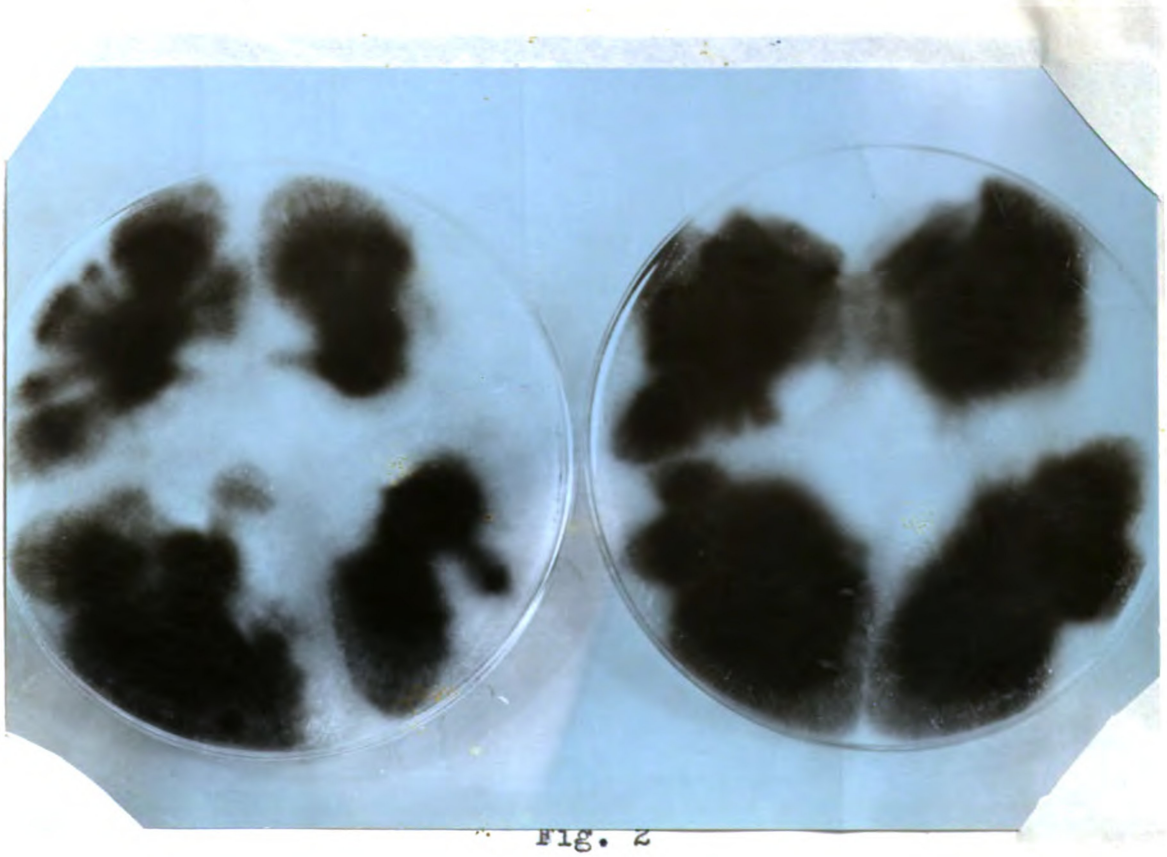


Fig. 2

Plate IX

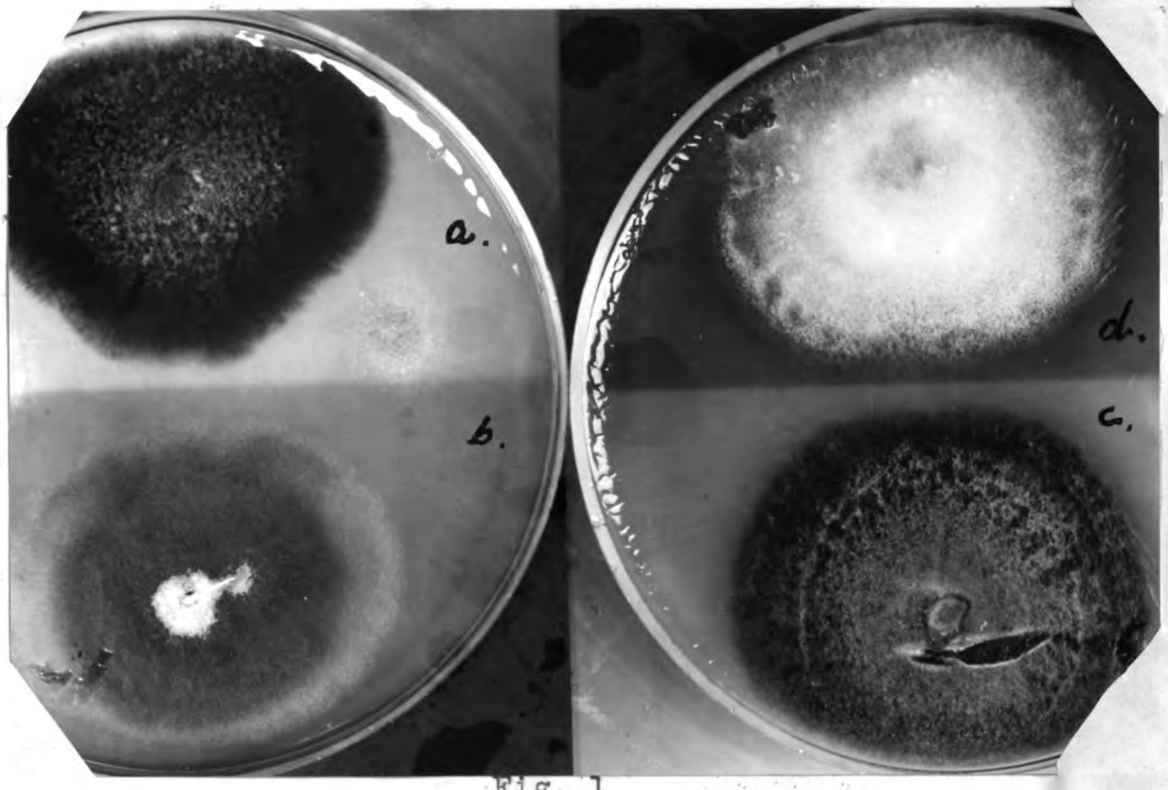


Fig. 1

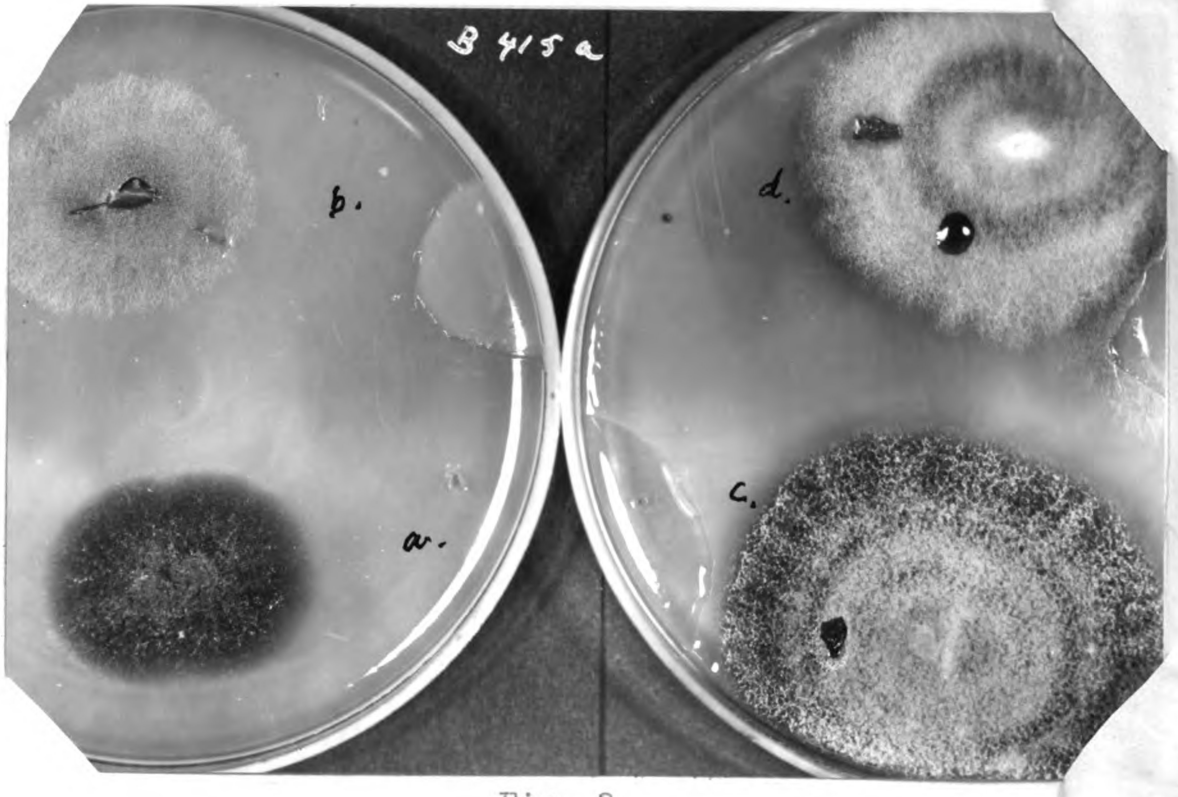


Fig. 2

Plate X

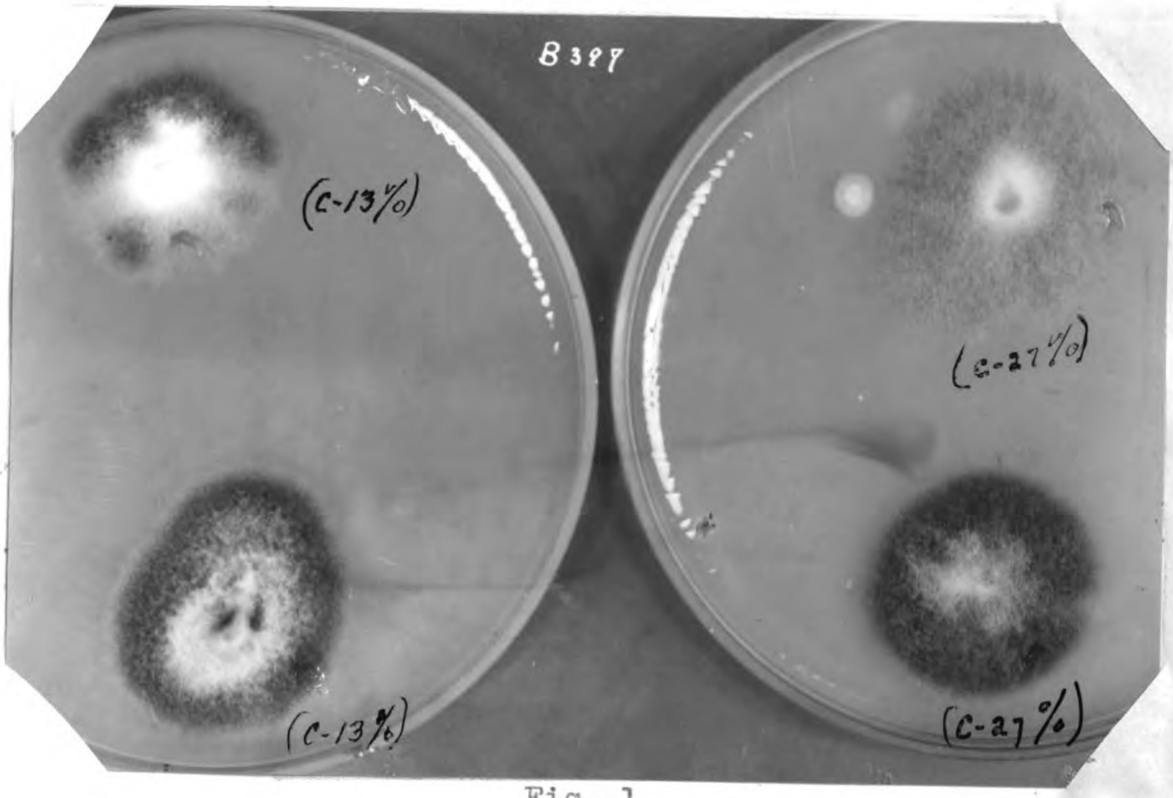


Fig. 1

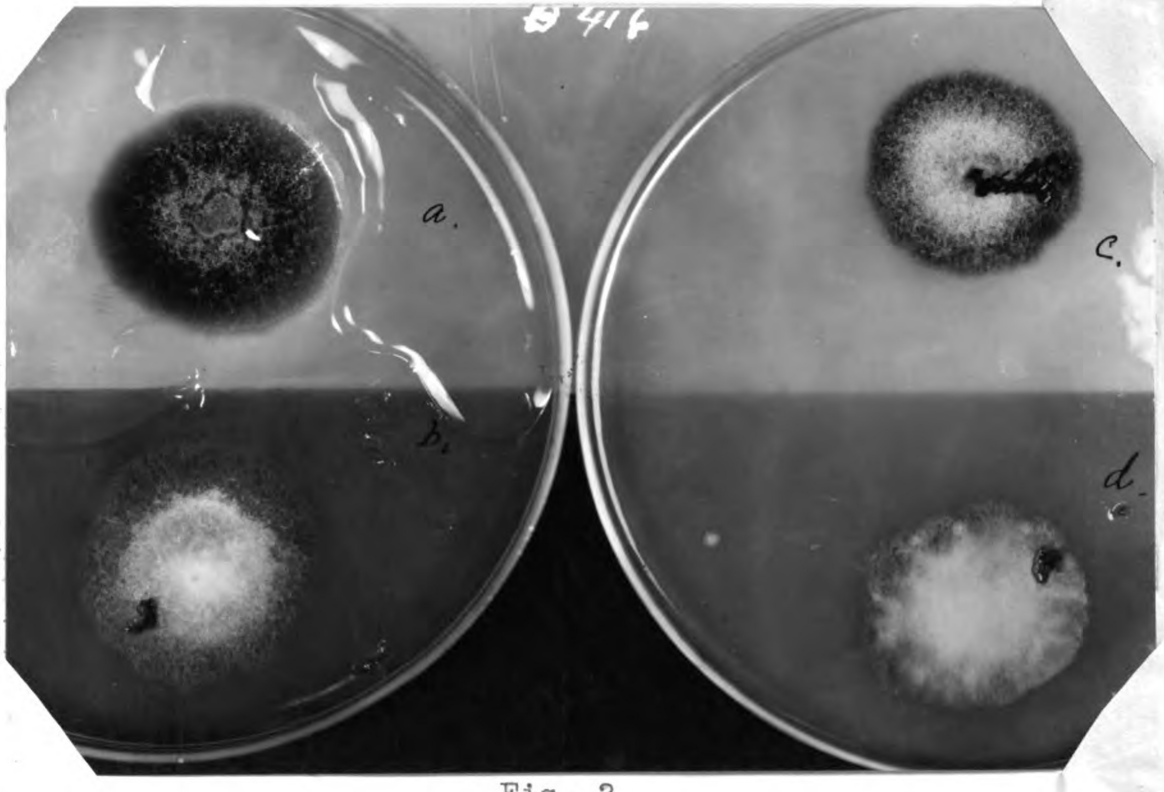


Fig. 2