

ACHROMATIC VARIATIONS

IN

PATHOGENIC FUNGI

Thesis for Degree of M. S.

Panos Demetrius Caldis

1925

THESE

Fungi, Pathogenic
Title

ACHROMATIC VARIATIONS
IN
PATHOGENIC FUNGI.

Panos Demetrius Caldis.

Presented to the Faculty
of the Michigan Agricultural
College as a thesis
in partial fulfillment of
the requirements for the
degree of Master of
Science.

January 1925.

THESIS

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A C K N O W L E D G E M E N T S.

The writer wishes to acknowledge his indebtedness to Dr. E. A. Bessey and to Mr. G. H. Jones, under whose direction this investigation was conducted, for valuable assistance and advice.

ACHROMATIC VARIATIONS IN PATHOGENIC FUNGI.

INTRODUCTION.

Variations in the cultural characteristics of fungi have generally attracted the attention of investigators, especially in later years when more careful study of the different organisms has been made. Ever since the value of growing the organism under study on different standardized media has been pointed out by Haulin (1888) (64)⁽¹⁾, Brefeld (1881) (17), H.K. Smith (1888) (26) and others, the effect of environmental factors has been carefully studied by Klebs (1886, 1888, 1913) (50), (51), (52), Leibinger (1911) (53), Coons (1913) (24) and others, the different factors, nutritional and otherwise have been carefully varied for the purpose of obtaining optimum development. Growing an organism on culture media selected at random is not conducive to best results and recent investigations by Young and Bennett (1922) (33) have shown that the conditions of best growth and development may vary within wide limits with different organisms.

Continuous cultivation of an organism on a particular medium probably not the best suited to it, also variations in tempera-

(1) Numbers in parenthesis refer to literature cited.

ture, moisture, light and other factors such as depletion of nutrients, accumulation of metabolic products etc. may or may not exercise an influence on its development as shown by Stevens and Hall (1930) (34), and variations not otherwise accounted for have frequently arisen. It has been noticed for some time that different cultures of parasitic fungi in the herbarium of Michigan Agricultural College were giving off tufts of white mycelium. It was for the purpose of gaining an insight into the cause of such variations that the present investigation was started. What are these white tufts? Are they mutants, environmental modifications or bits of sterile mycelium? How do they arise? Are their characters permanent? How do they differ from the parent strains? The time available did not permit an exhaustive study of all these questions but the little that was learned is reported here, together with a review of the literature available, with the hope that the information may be of assistance to some other investigator who may be interested in this fascinating problem.

REVIEW OF THE LITERATURE.

Variations in plants and animals have been quite frequently reported. The terms mutation, saltation, sporting, or variation are in general use. Authors, however, differ in the definition of these terms. Dobell (1912) (34) defines mutation as "those heritable modifications which have been induced in various ways in various microorganisms", while in a subsequent paper (35) he adds: "The word does not imply anything concerning the magnitude of change, its suddenness, or the manner of its acquisition. The term denotes a change in genetic constitution. Impermanent changes --not hereditary, are modifications.(Wolf-Saur)". Brierley (1930)

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(15) defines mutation as "a genotypic change in a pure line", while according to Almquist (1932) (1) it is "the formation of a new constant variety or species. Except hybrids probably all new species are produced by mutation." He further adds that most authors by mutation do not mean the sudden appearance of new constant varieties but simply a sudden change. Jordan (1917) (47) distinguishes three types of variation:

1. True Variation (Mutation).
2. Development of Latent characteristics.
3. Environmental Modifications.

He defines mutations as "changes due to intracellular changes brought about by non specific influences and not to direct adaptations to particular environmental conditions." By latent characteristics he means "dormant qualities in the organism manifested only in response to definite external influences." The environmental modifications are "closely related to the above class." They include "the effects of use and disuse" and are due to conditions offered by environment. Many so-called mutations are only environmental modifications which can be lost or retained for a long time.

He further stipulates the following requirements which must be fulfilled by every true mutation.

1. Appear suddenly without intermediate stages.
2. Be irreversible.
3. Change must take place in several characters.
4. Not all the cells of the parental strains are involved.

Pfeffer (1903) (61) distinguishes between variations that are termed indeterminate, spontaneous, saltatory or single variations and those that are designated as gradual, determinate, and adaptive. A saltatory variation involves the sudden appearance of some new

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property in one or few individuals and this is "... the result of prevailing conditions internal and external and it is readily comprehensible that unusual conditions should excite a tendency to saltatory variation and hence also to corresponding internal changes. That saltatory variations may be inherited is certain."

Stevens (1922) (23) uses the term saltation to cover variation in an organism whose cytological conditions and sexuality are unknown. This is a restriction of the meaning of the term as used by Pfeffer.

H. de Vries (1900) (97) in his monumental work on "the Mutation Theory" does not make any discrimination between the terms saltation, mutation or sport which he uses interchangeably. The Darwinian term "single variation" is used to mean the same thing. "Single variations are sporadic phenomena, appearing only from time to time and suddenly changing the forms of life. They cannot be induced at will, but must be waited for." (P.33) And in another place "....frequently they concern only a single character and then usually consist in the loss or latency of a character already present." (P.32) Mutations do not necessarily appear only once according to De Vries, (P.329) on the contrary he believes in the periodic mutability of species. On pages 247-259 he draws the laws of mutation which may be summed up as follows:

1. New elementary species arise suddenly without transitional forms.
2. They are absolutely constant.
3. Most of the new forms are elementary species and not varieties.
4. New elementary species appear in large numbers at the same time or at any rate during the same period.
5. New characters have nothing to do with individual variability.
6. Mutations are indefinite i.e. may affect all organs and take place in every conceivable direction.

7. Mutability appears periodically.

The majority of variations thus far reported in Bacteria have been forcibly induced by growing the different organisms on media to which normally injurious chemicals have been added. Dobell (1913) (35) in his definition of mutation does not take into consideration the "manner of acquisition" and in his paper on mutations in Bacteria he mentions several induced or spontaneous variations discussing them under two headings 1. Physiological and 2. Morphological mutations. Chamberland and Roux in 1883 (20) produced an asporogenous variety of Bacillus anthracis by adding 1/2000 Potassium bichromate to the culture fluid. These variations were constant even after passing the organisms through animals, and the virulence of the organism was not diminished. Roux (1900) (70) obtained similar results by adding a small amount of phenol to the culture medium and Phisalix (1892) (62) induced variations by cultivating the organisms at 42° C, or under various abnormal conditions. This asporogenous Bacillus anthracis was not less virulent than the normal strain and maintained the asporogenous characteristic under ordinary conditions of growth.

Phisalix (1892)(63) thinks that it is the combined action of the heat and the air and the slow oxidation of the protoplasm that produces the asporogenous strain. Rudimentary spores were formed in the cells of this strain.

Nigula (1897)(57) produced an asporogenous variety of Bacterium ramosum by continued cultivation in the presence of phenol while Villinger (1896)(96) developed a permanently **non-motile race** of B. coli and Wasserzug (1893)(39) a permanently motile variety of Bact. prodigiosus.

Hansen in 1900 (42) obtained asporogenous races of different Saccharomycetes by cultivating them at temperatures at which growth was still possible but at which spore formation did not take place. Varieties thus produced were constant. By adding small amounts of antiseptics he caused yeasts to increase their fermentative power.

Revis (1911) (66) grew Bacillus coli on peptone broth to which different quantities of malachite green had been added and thereby obtained a variety which did not possess the usual characteristic of producing gas from certain sugars. He repeated these experiments the following year with identical results, the lost powers not being restored by sub-culturing. The change was accompanied by a change in the appearance of the colony and the size and shape of the cells.

Almquist in 1922 (1) was able by drying and filtering to obtain new forms(plasmodia and microconidia) of B.typhosus, which, however, were not constant. Nevertheless he secured different strains of Spirillum cholerae by filtering, culturing and heating and he also obtained a filterable strain of B.typhosus which he called B.antityphosus. This last strain was kept under cultivation for eleven years, and it was never found to revert. It is evident, however, that in this last case as in those previously mentioned we are dealing with a sort of natural selection in which the organisms able to withstand the extreme conditions live over, manifesting their particular qualities once the overshadowing influence of the majority of the ordinary organisms disappears under the action of the dye, disinfectant or filter. This is particularly true since evidence from single cell

cultures is wanting, and rather doubtful in the early works. For example, inoculations of cultures in the works of Roux, Phisalix, Chamberland was made by adding a drop of blood from a sheep which died from anthrax.

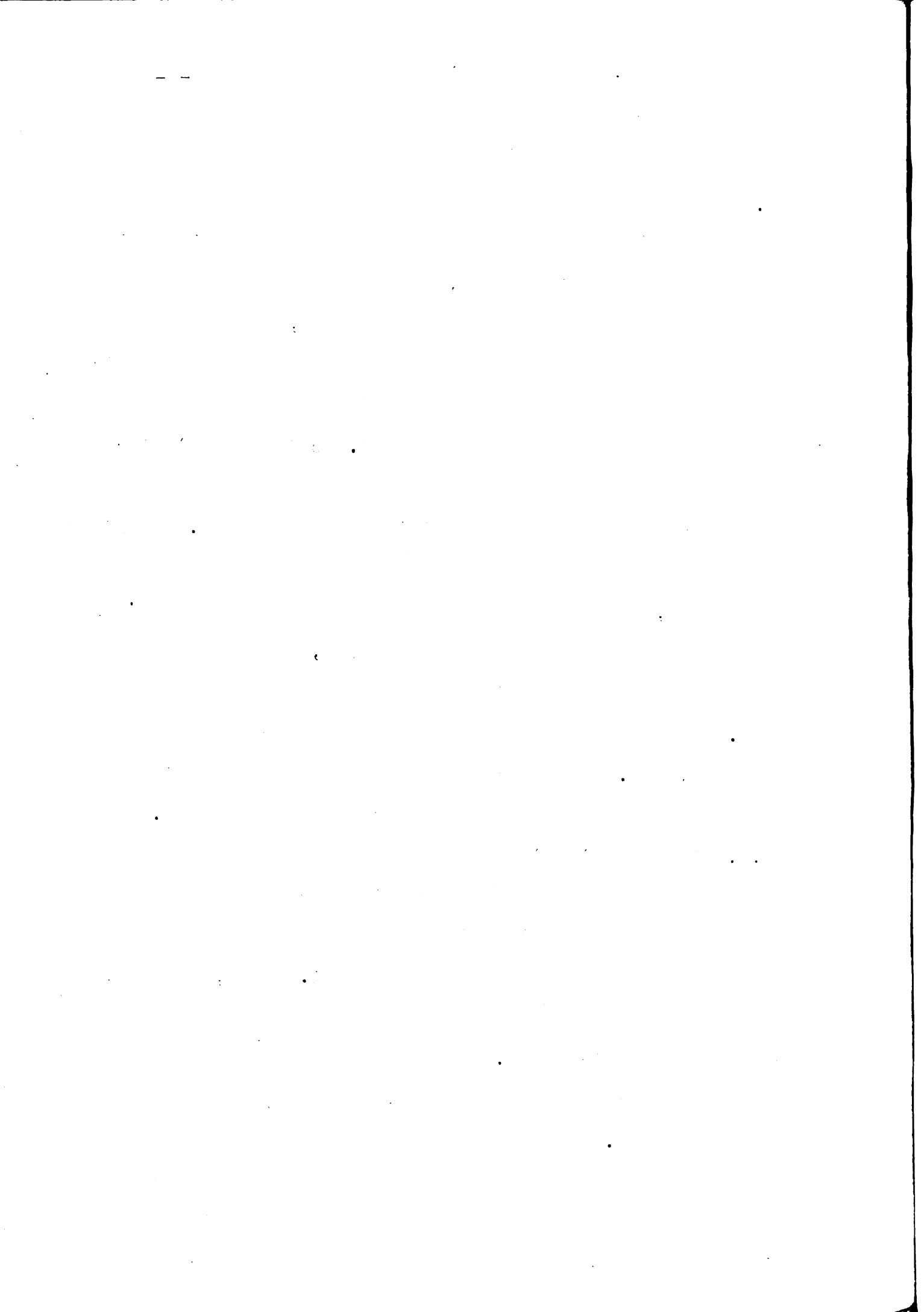
Variations induced by using similar methods have been obtained with fungi also. Brierley in 1920 (15) reports critically the works of Arcichovskij, Schiemann and Waterman. Arcichovskij in 1908 (2) obtained Aspergillus niger with yellow-brown spores by growing the ordinary forms on Raulin's fluid to which 1/1000 % $ZnSO_4$ was added. Schiemann in 1912 (72) obtained cultures of Aspergillus niger with chocolate-brown conidia or colorless turning to cinnamon colored, by growing the parent culture on media to which $K_2Cr_2O_7$ had been added in small quantities, or tall conidophored forms with varying forms of sterigmata, color and growth by exposing to high temperatures (40-42° C.). Waterman 1912 (90) produced different mutations in the way of changes in intensity of color, difference in number of spores and rate of growth by growing Aspergillus niger and Penicillium glaucum on 2% galactose, rhamnose or glucose, 1% boric and p-oxybenzoic acid or dichloroacrylic and salicylic acids. The variations were constant and true to form whether grown from single spores or mycelium.

Brierley (14) attempted a repetition of this work hoping to obtain similar results. As it might have been expected, however, he did not succeed, because, since the results of these, as well as of all similar investigations, depend upon certain fortuitous circumstances affecting the organism gerotypically and causing it to throw off permanent variations, it therefore was not to be expected that Brierley would be able to so exactly duplicate the great number of conditions as to produce identical results. He

reports phenotypic changes affecting the whole culture and which disappeared as soon as the organism was returned to a standard medium. Environment affects different individuals differently and unless one desires to doubt the good faith of these investigators there is ground to believe that, notwithstanding the universal nature and cosmopolitan study on these fungi, the particular strain used by each of these investigators was constituted genotypically in such a manner that it responded in its own particular way to different and variable external stimuli. Schieman (72) discussing the results obtained by her, observes that these variations do not appear as a response to a definite chemical used. A variation similar to the one observed on cultures where Potassium bichromate had been added, appeared under normal conditions on Pulst's medium at room temperature in one tube out of 173, while on 112 cultures with the addition of Potassium bichromate only three mutations appeared. Out of 397 cultures under the influence of stimulants of some sort, a 2.02% of variability was observed and in all cases only a few heads out of the many hundreds were affected.

E.F. Smith (1899) (20) reports variations in color, thickness of stroma and presence of perithecia in Neocosmospora vasinfecta and Steven and Hall (1909) (34) give a series of modifications due to environment in a great list of fungi. These, however, are not permanent variations and are simply what Jordan calls environmental modifications. Much more so since we have no record of their permanency and the cultures, probably, were not derived from single spores.

Edgerton (1906) (36) attempted to change the characters of *Gloeosporia* by growing them for 3 months on Elfving's nutrient solution or on potato agar to which various substances had been



added e.g. 10% glucose, 1:100,000 copper sulphate, or a drop of lactic acid. Frequent transfers on the same kind of medium were made, but the variations induced in this way were not permanent. When, however, the organisms were grown for a greater length of time on the same type of media variations in the amount, nature and color of the aerial mycelium, color of the substratum, and the presence or absence of postules were observed, and these variations did not disappear with the age of the colony.

Blakeslee in 1913 (7) induced mutations in *Mucors* by chemical and other forms of stimulation. In 22971 colonies he reports 25-30 types, the aberrant characters being absence, increase or decrease of zygosporangia production, peculiarities in color, density and rapidity of mycelial growth, difference in height of sporangial filaments, the almost exclusive production of yeast-like cells and the production of filamentous mycelial growth devoid of sporangia. Some of these are temporary conditions while others are permanent, the characters being reproduced for several sporangial generations. The same author reported in 1920 (3) a change in sexual activity tending toward neutrality in both the plus and minus races of *Mucor mucedo* by growing them for several generations at unfavorably high temperatures. This condition is not permanent, however, and the sexual activity is regained in a few generations of cultivation at low temperatures. He concludes that "the apparent neutrality may mean merely that we have not yet happened to expose these forms to the peculiar environmental conditions necessary."

The variations reported above have been generally induced through exposure to unfavorable environmental conditions. Variations, however, do not depend entirely on changes in environment,

nutrition etc. but may be brought about through inherent properties of the organism itself. These are the so-called spontaneous variations and there has been a great number of them reported by different investigators. Gradual adaptive variations do not naturally fall into this class as the factor of natural or artificial selection is in operation in such cases as well as in those variations induced by the use of disinfectants, dyes, heat etc.

Rettger and Sherrick in 1911 (65) reported several instances of variations obtained by selection of organisms for a certain property as e.g. the work of Goodman (1903) (39) on B. ^{acidus} diphtheriae. He was able to obtain high and low acid producing types after 36 selections by simply growing the organism on 1% dextrose bouillon tubes and selecting for high and low acid production. Scheurlen (71) in 1906 was also able to obtain colorless growths of Bact. prodigiosus by growing the organism on potato and selecting the least colored portion of the growth for subsequent transplants. Rettger and Sherrick (65) were able to select a white and red strain from a culture of Bact. prodigiosus which was weak in chromogenic properties. These strains were carried for sixty five transfers and were found to be constant. There was no modification or other change in environment except the fact of selection. A similar attempt on a culture of B. ruber balticus failed. Buchanan and Truax (1910) (17) also failed to establish high and low acid producing strains in Streptococcus lacticus. A strongly chromogenic strain of B. pyocyaneus was also developed by Rettger and Sherrick (65) from a strain which had almost lost that property and had been neglected for a long time having been allowed

to dry down. In this case we have a natural selection of the type most resistant to drying which also happened to be the one possessing the best chromogenic properties.

Coming now to variations which could be truly classed as spontaneous, the work of Beijerinck 1897 (6) should be first mentioned. He isolated from *Schizosaccharomyces* sp. different strains differing in color - white, brown or intermediate - which also differed in spore production, the brown strain lacking this property. The white and the brown strains were constant, but the intermediate showed variations. Jorgensen in 1903 (42) isolated races of yeasts from single spore cultures which showed variations in brewing qualities. Hansen (43) 1900 succeeded in obtaining asporogenous types of *Saccharomyces pastorianus*, *S. ellipsoideus*, and *S. cerevisiae* by plating and selection. Massini (1907) (55) found in pure cultures of *S. coli mutabilis* forms that would ferment lactose, and grow red on Indole's medium. This character was permanent. The parent strain was an ever sporting variety, the variations remaining constant. Massini's work was repeated by Burck (1908), Sauerbeck (1908), Bercke (1908), Kowalenko (1910) and many others.

Barber in 1907 (3) made selections of individuals of *Saccharomyces anomalus* varying in form and size. He was able to obtain variations, independently of conditions of cultivation. These variations consisted in the appearance of abnormally elongated cells which adhered in groups and also in the partial loss of sporogenesis. These morphological characteristics persisted in a great variety of media. Physiological characters such as greater resistance to **desiccation** and high temperatures, and higher fermentative action were possessed by the new race in more marked degree than by the parent. He also worked with ^{Ac. 44-3} *S. coli communis*. By his inde-

nious method he selected ^asingle organism and was able to isolate new races which were characterized by a tendency to grow into long filaments and by loss of motility. Such races were constant. Similar experiments with B. typhosus failed to give constant variants, the same being true of attempts to develop asporogenous B. megatherium. Jordan in 1915 (47) obtained from a single cell bacterial strain, a strain of B. coli which possessed the power of fermenting sucrose and raffinose and of producing gas. The variation fulfilled all the requirements postulated by him: i.e. it appeared suddenly without intermediate stages, was irreversible, changes appeared in several characters, and involved only a part of the cells of the parent strain. The strain remained permanent for over 500 generations.

Among fungi the earliest recorded sudden variation was reported by Edgerton in 1908 (36). While plating a culture of Glomerella rufomaculans he found two distinct forms, one producing perithecia abundantly and singly, while the parent form produced perithecia in modules scattered over the medium. The perithecia were identical but the asci and spores of the variant did not mature. It grew slowly on apple. He concludes "Mutations so far as known by the writer have not previously been recorded among fungi, but the form just described seems to be one without question." In a later paper on plus and minus strains in the genus Glomerella (37) he believes that the variation mentioned above "was really the minus strain of the bitter rot fungus". He further remarks: "The Glomerella forms show so much variability that it is possible that we have in the cultures that have been described merely isolated variation that may not be

common in the Ascomycetes as a group or even within the genus *Glomerella* itself." Shear and Wood in 1913 (76) in their monograph on the genus *Glomerella* make notice of the variability of the genus "no character seems to be well fixed. Appearance of mycelium is rather uniform. Sclerotia, setae, elongated spores, perithecia, asci, ascospores, and paraphyses vary extremely in number, size, shape, way borne, lack or presence, abundance, arrangement or location." They could not trace any relation between these variations and environment and seem to conclude "that variations are due to internal cause."

Burgoff in 1914 (13) reported variations in single spore cultures of *Phycomyces nitens* in many characters. Crabill in 1912 (23) noticed four distinctly different types of *Phyllosticta pyrina* while conducting pure culture studies by the poured plate method on this species. The variations were in the way of differences in color, presence of aerial mycelium and abundance and distribution of pycnidia. He regards these types as possibly elementary species in the LeVriesian sense or pure lines according to Johanssen's use of the term. In a second report in 1913 (22) he adds that these four strains were similar microscopically and remained true to type from generation to generation. In a subsequent note in 1914 (20) he reports an asporogenous mutation in the same fungus occurring in a pure (petri-dish) culture. The mutant under special stimulation produces only rudimentary fruiting bodies but not spores. Crabill in 1915 (31) reported a case of dimorphism in *Coniothyrium pirinum* (Sacc) Sheld. Evidently according to Sheldoh (77) we deal with the same fungus *Phyllosticta* which he transferred to the genus *Coniothyrium* in 1907. Kutto and Pollacci in 1915 (50) however, carrying compa-

relative investigation of both forms report that the species Phyllosticta pyrina should be maintained because it is distinctly different from Coniothyrium pyrinum. The conidia of both forms are hyaline at first but those of P. pyrina turn black while those of C. pyrinum turn darker. That Crabill calls a virid strain was thrown off from different single spore cultures of the organism. Selection of a abnormal environment were not a factor in the development of this virus, sporically fruiting strain, and he concludes that "the virus strain is a mutant arising at irregular and unpredictable intervals". The virus strain was also isolated from nature as well as several times from these plus strains of the organism where it appeared as a variation or mutation. Both strains were pathogenic. Autto and Pollacci in 1929 (60) found that the different media influence the dimensions of spores and intensity of color of Coniothyrium tinolense Bubak (considered identical ^{with} ~~to~~ Coniothyrium pyrinum (Sacc.) Sheld., the latter being a synonym (59)). Phyllosticta pyrina assumes the characters of Ascochyta viz: septate spores of weak color on beer wort gelatin and lemon gelatin neutralized with sodium hydroxide. These characteristics remain constant in subsequent generations on the same media but are lost on other media.

Brierley in 1929 (14) reported a sudden variation in a single conidium culture of Botrytis cinerea. The variation was in the way of a colorless sclerotium produced once along with 24 black ones in a transfer culture derived from a single conidium. The variation appeared suddenly, without intermediate stages, only once in the history of the strain and of the species, as far as it is known, and has been constant for a great number of generations. The variation is only in one particular

character -- that of the colorless sclerotium, and there were no differences between parent and mutant in other morphological or physiological characteristics. An extensive and critical study of the origin of the variation was made with the conclusion that this variation cannot be called a mutation on account of the uncertainty of the purity of the species, the explanation of the colorless form lying in "the extremely rare chance of a fertile conidiophore arising from a cell contaminated by the nuclei or cytoplasm of a phenotypically different individual."

Blakeslee in 1930 (3) reported several variations in a pure culture of Necor caneyensis which was kept running in vegetatively propagated pure lines for 19 years. The variations were in the color or in the compactness of growth of the mycelium, in the length of the sporangium stalks or in the size and abundance of the zygospores, or more commonly in the reduced size of the colonies. These mutations remain constant but eventually they revert to the normal type. Two of them, however, were more stable than the rest. One is a dwarf type which remained fixed through seven years culture on many kinds of media. The colonies are small, lacking sporangia and zygospores. The other mutant lacked zygospores and was grown for seven years through 16 non-sexual generations but it was occasionally found to revert.

Dastur in 1930 (32) reported variations similar to those recorded by Edgerton (36) in Gloeosporium piperatum E & E. (Glomerella circulata (Sten) Sp & van Sch.). These variations consisted in the absence or presence of perithecia, acervuli,

setae, and development of aerial hyphae, and were found to be permanent. Other variations in the way of sterility were found to revert when the organism was passed through its host. This reversion, however, was ultimately lost. In 1921 Burger (19) reported several variations in Colletotrichum gloeosporioides. From single spore cultures, strains differing in color were obtained producing spores: A black and a white strain was isolated. "These mutations resemble the strains isolated from natural environment."

Lee Bonar in 1922 and 1924 (11)(12), reported an albino mutation in Brachysporium trifolii from single spore cultures kept growing for three years. The variation started as a white sector in a petri dish culture, produced spores as did the parent and was carried through sixteen non-sexual generations both vegetatively and by single spores, always remaining identical with the parent except for the color. Sudden variations in fungi were reported by F.L.Stevens in 1922 (33). In connection with a study of the Helminthosporium foot-rot of wheat he noticed that, occasionally, certain sectors of a colony growing on an agar plate differed more or less from the rest of the colony. These variations were found persistent ^{and} constant. The variants differed in density of color, rate of growth, low number of conidia produced, length, breadth, color, septation, thickness and shape of conidia. The variants were permanent even through the conidia.

A. Blochwitz (10) in 1923 obtained from poured gelatin plates of Aspergillus versicolor a blue colored strain which remained constant for over forty generations. A study of the

pigment in the species revealed that the mutant lacked color in the walls of the conidia and the mycelium. The variation appeared only once.

The latest report of sudden variation in fungi was made by H. Chaudhuri in 1924.(21) A saltation in Colletotrichum biologicum n.sp. is described as having appeared once in a strain kept under cultivation for four months. The saltation appeared on an Oat Meal Agar culture and consisted of pink mycelium bearing large sclerotia in concentric rings and small in radiating lines while the parent has only large sclerotia. The saltation was constant for over two years on Cocons' medium or Oat Meal agar but it invariably reverted on being grown on Potato agar. The saltation produces a pink color which turns nearly black with increase in temperature.

EXPERIMENTAL WORK.

The investigation was started in February 1922. The following fungi which were known to give off variations in culture were transferred on Clinton's Oat Meal Agar (23) and Shear & Wood's Corn Meal Agar (73): Sphaeropsis walleyana Pk(Exp. Sta. No 125), Colletotrichum linduthium (Sacc.&Magn.) Br. & Cav. (Strains I & II), and Cladosporium fulvum Cke. The isolations were made from both the normal looking portions of the culture and from those portions that exhibited peculiarities in appearance. Septoria api Br. & Cav. was isolated from diseased celery leaves by the modified poured plate method to be described later.

Methods of Investigation. Although the parent strains were proce-

nies of single spore cultures, in order to make sure that only single spore cultures were used in these experiments, plates were poured by the ordinary dilution method using very dilute suspensions of spores in Corn Meal Agar. As soon as a well isolated spore was found to have germinated, usually on the second day, its location on the plate was marked by an ink dot on the back of the petri dish and immediately the agar lying over the dot was carefully scooped out with a flattened sterile platinum needle. The agar containing the germinating spore was placed on a sterile slide and carefully examined with the high power of the microscope to make sure that nothing ~~else~~ but the single spore had been removed from the plate. This requirement once satisfied the tiny piece of agar containing the spore was again placed on a corn meal agar slant and allowed to grow. Of among more than twenty isolations made by this method there were a few that did not grow. However, none was found to have been contaminated either by bacteria or fungi in the process of transfer.

In this way single spore cultures on Corn Meal Agar and Oat Meal Agar slants of Cladosporium fulvum Oke, Strains I & II of Colletotrichum linderothianum Br. & Cav., Septoria apii Br. & Cav. and Sphaeropsis mal/orum Pk were obtained. The plan was to grow these organisms on a variety of media and watch for variations such as white tufts of mycelium, or any other peculiarity of appearance..No chemicals, disinfectants or dyes were added to the cultures neither were the temperature, moisture, light or other factors modified except in special experiments.

In the course of the investigations the following media were used, prepared according to directions given by their originators. Corn Meal Agar (76), Oat Meal Agar (23), Difco

Prune Juice Agar prepared by the Digestive Ferments Co of Detroit Mich. (26), Nutrient Broth Agar (23), Coons' synthetic Agar (24) Richards' synthetic agar (27), Leonian Synthetic Agar, the latter being essentially Coons' synthetic agar with 5 times as much N/5 Asparagin per 100 c.c. of medium, Potato and Carrot Plugs, Corn Meal in 100cc. Erlommeyer flasks (75) prepared by adding a teaspoonful per flask with 4 times as much water, autoclaving at 20 pounds of pressure for 15 minutes and finally **rice flasks** prepared in the same way.

The chemicals used were "Baker's Analyzed", and the glassware was either Pyrex or Jena. All glass culture dishes were carefully cleaned by immersing overnight in cleaning solution, followed by four rinsings in tap water and one rinsing with distilled water. The water used was distilled water which had been redistilled in a block-tin still or in special cases (**PH** experiments) in a Jena Glass still.

APPEARANCE OF VARIATIONS. The appearance of variations will be discussed for each fungus separately.

Septoria petroselinii var. apii Br. & Cav. was isolated from diseased ~~celery~~ plants where it causes leaf and stalk spotting, the disease being known as **Late Blight** ^{of} **Celery**. Beach in New York (1923) (5), Rogers in California (1911) (69), Coons and Levin in Michigan (1916) (27) and several others have worked with this disease of celery but have not described the growth and behaviour of this fungus on different media. Six single spore isolations were made on Oat Meal Agar and transferred on Corn Meal Agar, Prune Juice Agar, Leonian Synthetic Agar, Carrot Plug and Corn Meal Flask. The organism grew normally on all these media for

a week forming a black dense growth with numerous pycnidia scattered over the surface of the medium. Ten days after inoculation a white tuft was found in strain No 5 on Corn Meal Flask, forming a striking contrast with the normal black growth. (Plate I, Fig. 1a) Attempts were made to transfer this white mycelium to Richards Preparation Dish, Oat Meal Agar, and Corn Meal Flask but it invariably grew jet black in the new culture.

The same strain gave a second white tuft in a culture on Prune Juice Agar which was transferred from a Leonian Agar Tube. The white tuft was transferred back to Leonian medium, but it grew black again. Strains 1 and 2 behaved in a similar way. Strain 1 threw white variations on Oat Meal Agar and Leonian Agar but both these reverted when isolation was attempted. Similar variations of Strain 2 on Oat Meal Agar and Leoniaⁿ Agar reverted on being transferred.

Sphaeropsis mal/orum Pk was transferred from the Mich. Agr. Coll. Experiment Station stock cultures where it was known to behave e^ra^atically. There was some difficulty experienced in obtaining a single spore culture of this fungus since it would not fruit on Corn Meal Agar or Oat Meal Agar. But on being grown on Coons' Synthetic Liquid^{in a} preparation dish it fruited abundantly and two single spore strains were secured from the dilution plates. These strains were grown on Oat Meal agar, Prune Juice Agar, Corn Meal Agar, Corn Meal Flasks and Rice Flasks. The organism grew luxuriantly, producing an abundant aerial growth of a greenish blue green tinge. The mycelium at first was cottony white but later turned dark or even nearly black.

White tufts appeared on Corn Meal Agar but on being transferred to Leonian Agar gave rise to a dark gray growth with

several black pycnidia. The strain was then discarded. Hesler (43) in 1916, in describing the cultural characteristics of the Black rot fungus on different media, says: "Colonies are at first cottony, after 2-5 days the submerged threads are green or blue green for about a week, after which the growth is dark or even black, but almost without exception the outer ends of the aerial thread maintain their original cottony appearance." Brooks and De Merritt in 1912 (16) describe three strains of Spaeropsis mal/orum ~~PK~~ which differed markedly in shape of pycnidia and spores as well as in their pathogenicity. They do not mention, however, any other cultural characteristics. Scott and Rorer in 1908 (73) and the numerous other investigators who have worked with this fungus do not describe the cultural characteristics. Clinton (32) attributes the black color of the rot of apples to the mycelium of the fungus "which while at first hyaline eventually becomes dark olive."

Colletotrichum lindemuthianum (Sacc. & Magn.) Br. & Cav. Exp. Sta. Strain I., was also transferred from the M.A.C. Exp. Sta. stock cultures. Dilution plates were poured and with the precautions described, two single spore isolations were made. They were grown on Corn Meal Agar, Corn Meal Flask, Oat Meal Agar, Leonian Agar, Prune Juice Agar, Rice Flasks, Potato and Carrot Plugs and in all cases the organism grew normally. The mycelium was colorless at first but it soon turned black and the pink spore masses were scattered in small pustules, 1 mm. in diameter, on the surface of the mycelium, which grew within the substratum. The following are short notes on the appearance of

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this fungus on the different media used . There were no differences between the two single spore cultures from Strain I.

Leconian Medium. Thin colorless mycelial growth. Acervuli and pycnidia. No discoloration of substratum.

Prune Juice Agar. Abundant black growth, covering entire surface of slant. Blackened substratum. Acervuli scarce.

Oat Meal Agar. Abundant Black growth covering entire surface of slant. Blackened substratum entirely covered with acervuli.

Rice Flasks. Abundant black growth covering the surface of medium and extending deep into the substratum. Deep mouse gray and dark olive gray aerial growth with scanty white tufts. No spore masses.

Corn Meal Flasks. Surface covered with black growth. White tufts. Acervuli very abundant. No aerial mycelium.

Carrot Plug. Black growth covering entire surface. No spore pustules.

The characteristics of growth as given above compare well with those described by Beach (4) in 1893, Scribner (74) 1887 and others.

White tufts appeared on the Corn Meal Flask and Oat Meal Agar slants within 20 days after inoculation but transfers from these on Corn Meal Flasks and Oat Meal Agar slants grew perfectly black. Other attempts to be described later (p.36) also failed to establish a white strain.

Colletotrichum linderothianum (Sacc. & Magn.) Br. & Cav.
Exp. Sta. Strain II. This strain was known to be more persistent in variability. A transfer from the Exp. Sta. stock culture was made on Corn Meal Agar and on Coons' solution ^{in a} preparation dish. No spores were found on the second and in dilution plates great difficulty was experienced in making single spore isolations on account of the lack of spores.

Five single spore isolations were made and the organisms grown on different sets of media as described for strain I. The growth was ~~typical~~ ^{THE SAME} in both strains and the descriptions given of the growth of strain I, on the different media, apply equally well to strain II. Far more white tufts appeared, however, especially on Corn Meal Flask and Oat Meal Agar and these variations proved to be constant. The following is an account of their appearance. Single spore culture No 5 on Corn Meal Flask produced white tufts of mycelium 20 days after inoculation. A little of the white mycelium was transferred on Oat Meal Agar, Leonian Agar, Prune Juice Agar, Corn Meal Agar, Corn Meal Flask and from these fifteen subcultures were made throughout the summer on a variety of media, the fungus growing perfectly white and forming a rather compact mat with very little aerial mycelium on the surface of the Corn Meal or a rather slimy layer on the surface of Oat Meal Agar slants. The strain was never seen to revert. Plate II (fig. 2a & b) shows two cultures of Colletotrichum lindemuthianum Exp. Sta. Str. II, Fig. 2a shows a culture having thrown off white tufts while fig. 2b shows a normal culture. Plate III, fig. 1 shows a culture of the white form.

Single spore culture No 5 threw off a similar variation on Prune Juice Agar which proved also to grow normally, through several generations and on different media. It never reverted. The other single spore cultures were not seen to throw off such variations.

Cladosporium fulvum Oke . Four single spore cultures were secured through dilution plates poured from a transfer of this

organism from the stock culture of the Experiment Station. This fungus causes a leaf mold of tomatoes and it was studied extensively by Makenson (54) in 1913 in this laboratory. In Makenson's paper no mention is made of any variations appearing in cultures except the production of a purple color on certain media. This fungus has a tendency to grow into small cushion like clumps of velvety olive green color.

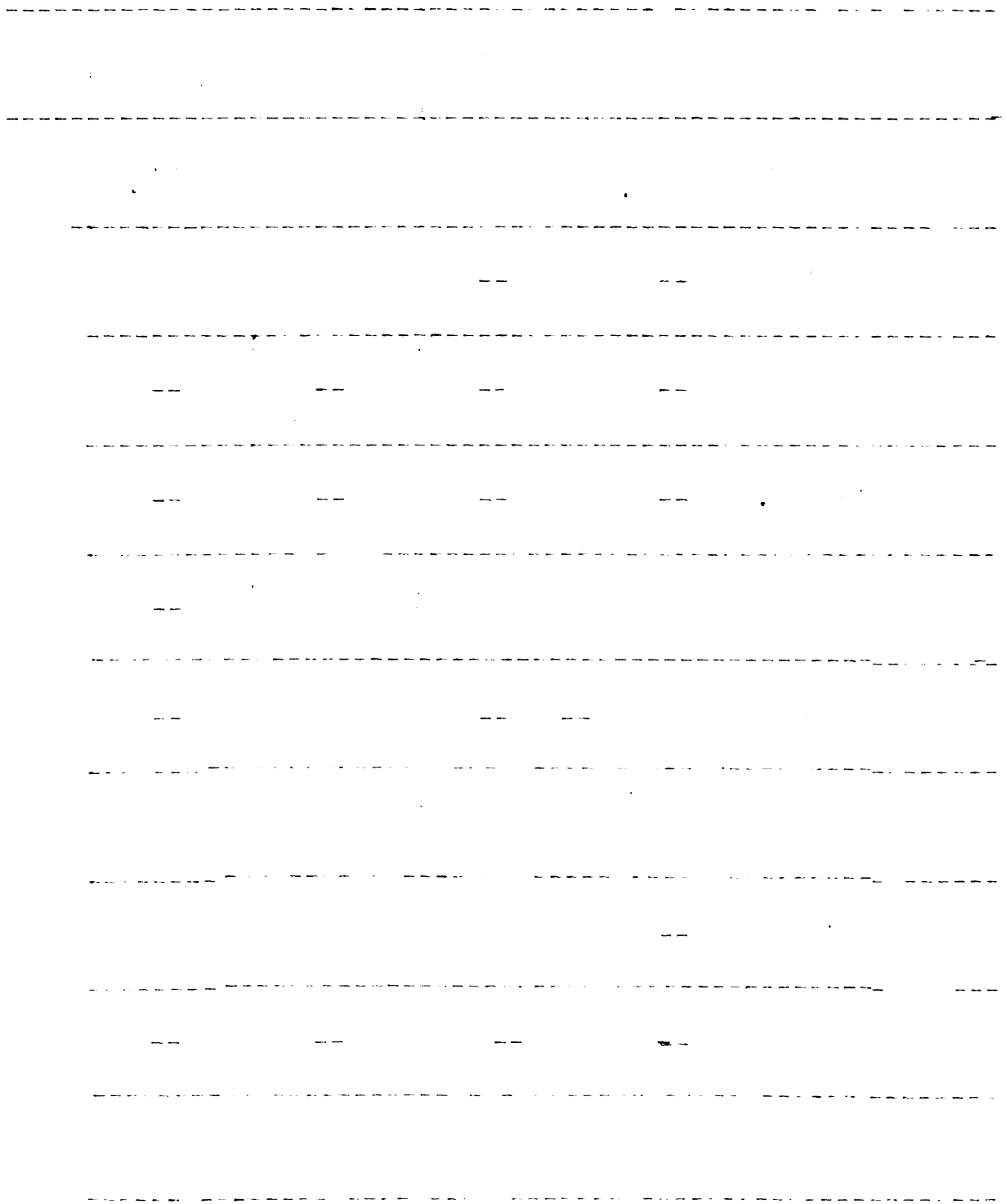
The four single spore cultures were transferred on Corn Meal Flasks and in about 20 days strain No. 4 showed a small white colony arising from the center of a thick mass of small ordinary olive-green colonies. A picture of the Flask was taken (Plate I, Fig. 1¹~~8~~) but an attempt at that time to transfer the colorless hyphae ^{to} Corn Meal (Flask) and Oat Meal Agar failed presumably because brown spores were taken along with the white mycelium. When, however, the white hyphae were allowed to grow again in the original flask, it was possible to obtain the white type, uncontaminated by the parent strain, on Prune Juice Agar, Leonian Agar, Corn Meal Agar and Oat Meal Agar. It has been grown since for two years on a great variety of media and it has never reverted. The list of media includes besides those mentioned above: Cocons Synthetic Agar, Corn Meal Flask, Rice Flask, Raspberry leaf agar, Nutrient Broth Agar, Czapeck Synthetic Agar, and Lima Bean Infusion Agar.

Besides strain No 4 the other strains threw off white forms at different times and in many case successful isolations were effected. Table I presents an account of the appearance of these variations in the different strains and on the different media used. The different white forms from the different single spore cultures are identical and have been used in the various experi-

ments . The so called white forms are not perfectly white but have a pink or lilaceous tinge which is carried by the hyphae.

TABLE I. The appearance of achromatic variations in four single spore strains of Cladosporium fulvum Cke otherwise identical, on a series of standard media. Positive cases marked: **, Negative --.

MEDIA	Strain I	Strain II	Strain III	Strain IV
Corn Meal Agar				**
Corn Meal Flask	--		**	**
Rice Flask	--		--	--
Prune Juice Ag.	--		--	--
Oat Meal Agar	**	**	**	--
Leonian Agar	**	--	**	--
Coons Agar				**
Richards Liquid	--			
Carrot Plug Potato Plug	--	--	--	--
Nutrient Agar				**



COMPARISON OF PARENT AND VARIANT OF CLADOBATHUS FULVUS CKE.

Wakeman in 1913 (54) and Snyder in (55) in 1924 describe the growth of Cladobathus fulvus Cke both on the parent and on different culture media, giving numerous details of the mycelium, conidiophores and conidia. The parent strain used in these investigations was compared with the descriptions of the above mentioned authors and was found to correspond exactly to those descriptions. **Plate II** gives several detailed drawings of the mycelium, conidiophores and conidia of the parent and variant forms. The drawings were made from a two month old culture of Czepeck Saxtress agar, the material taken from the growing edge of the colony.

No differences can be seen between the young growing mycelium of the parent and of that ^{of the} variant. The mycelium is about 2 μ wide, hyaline, quite delicate and of irregular septation, branching at a right or 45° corner angle. The cells contents are rather granular. The aerial mycelium of the variant is much wider 3-4 μ , very granular, and irregular, with occasional inflations resembling chlamydospores. Such mycelium with the exception of the color and the uneven septation resembles the conidiophores of the parent form, but no conidia or other similar structures were ever seen. The inflations referred to above have been described by Wakeman (54) in the parent strain on mycelium from Corn Meal Agar cultures. The parent form produces an abundance of typical conidia.

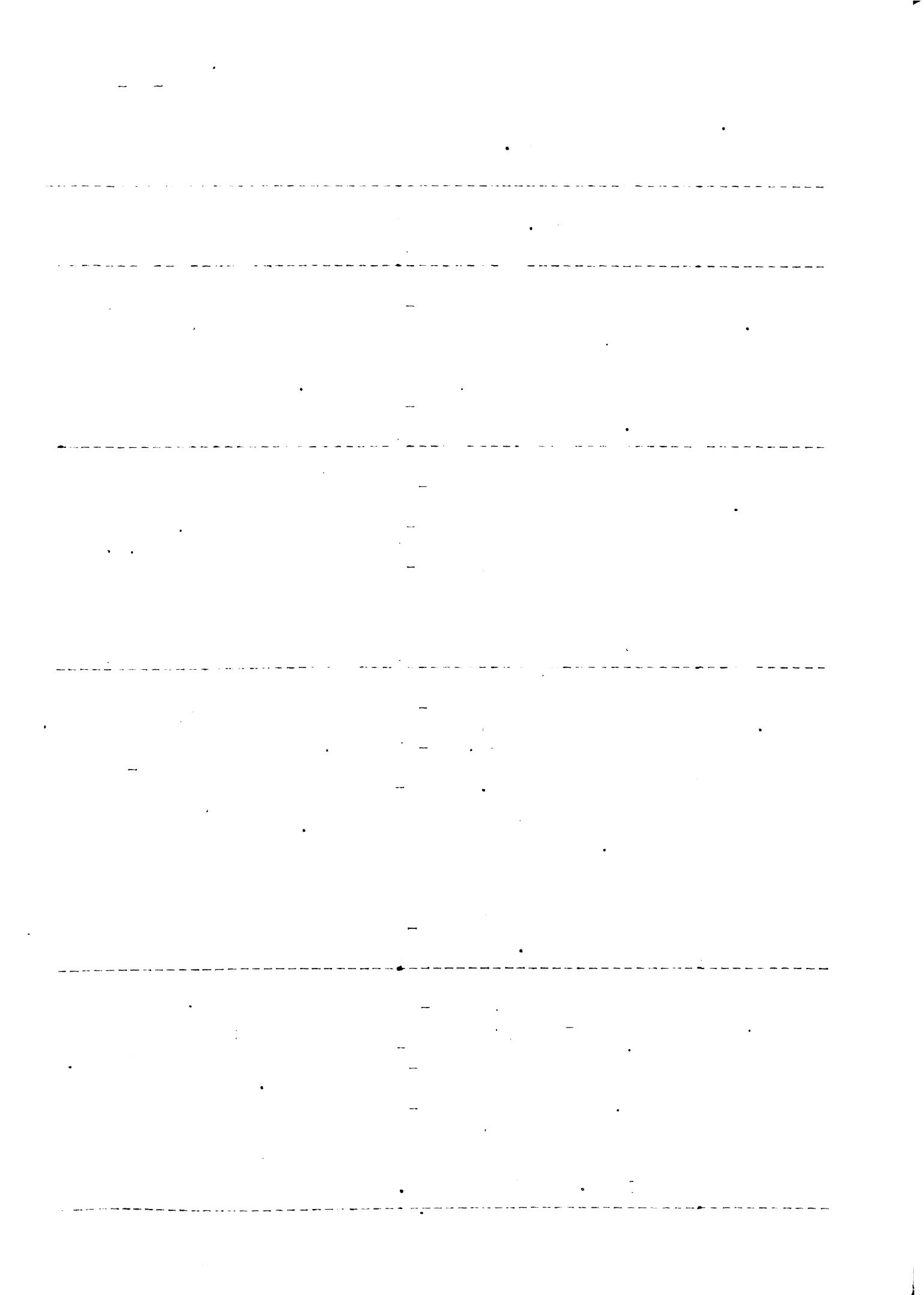
The ~~only~~ ^{striking} difference between the parent and the variant, therefore, seems to be the lack of fruiting in the variant. The mycelium of the parent form is hyaline and the olive brown appearance of the culture seems to be due to the conidia and

the conidiophores and this explains the white appearance of the variant. The purple or pink color which has been observed on certain media (usually Corn Meal Agar, Prune Juice Agar, Czapeck Dextrose Agar) seems to be located partly in the mycelium and partly in the conidia and the conidiophores. This color has been very often observed in the mycelium of the variant form and gives to the colony the hues described later. ~~ethan-ethanol~~. Stewart and Hodgkins (25) in 1923 report a similar behaviour as to color production by Sporotrichum rose ~~PK~~. This fungus produces a bright red color whenever the hyphae come in contact with sugar beet, turnip, potato, wheat, bread, cabbage and apple. Color did not appear on potato agar, alfalfa agar, alfalfa stems and clover stems. The red color appears in these media irrespective of conditions of light, temperature, alkalinity or acidity not incompatible with growth. It was, however, more bright on acid media and in darkness. With the fungus under investigation the pink coloration appears more readily on alkaline media (page 56) and although the nature of this pigment was not investigated it appears only on certain media rich in sugars and seems to be affected by physical factors such as acidity etc.

Table II presents a comparative description of the growth of the parent and variant forms of this fungus on the different media employed during the investigation. Ridgway's Color Standards (63) were used for description of colors.

TABLE II. Growth and pigmentation of parent and variant on different media.

Medium	Parent.	Variant
Corn Meal Agar.	Moderate growth, abundant spore production, Euclidian brown to olive brown surface color, with a dark livid purple ring through the medium.	Moderate bushy growth, no spores produced. Perfectly white, with a faint aster Purple color through the medium.
Corn Meal Floak.	Abundant growth covering entire surface of medium. Tufts of colorless mycelium arising from dense parts. Abundant spore production of same color as surface which is a brownish brown tint.	Moderate growth in tufts bunches. Pure white. Mycelium hyaline. No spores. Plate III Fig. 3.
Oat Meal Agar.	Abundant growth covering entire slant. Very dense sporulation. Mental breeze to buffy citrine in color. Extensive white aerial growth tinged with pink or brown yellow. Pigment through medium retains black with auburn edges towards the end of the slant, and purplish brown unpigmented medium.	Moderate growth. Pure white or tinged with vinaceous pink. Growth in medium burst like but no diffusion of the pigment through medium. No spores.
Prune Juice Agar.	Abundant growth. Colonies 1/4 - 1 cm. in diam. Agars brown in color with occasional honey yellow or white tufts. Spores in abundance, same color. Growth through the medium retains black, diffusing for 1 cm. around colony.	Moderate growth. Surface of colony Pale Pinkish Buff, the medium colored plainly Dusky Lull Violet. No spores.



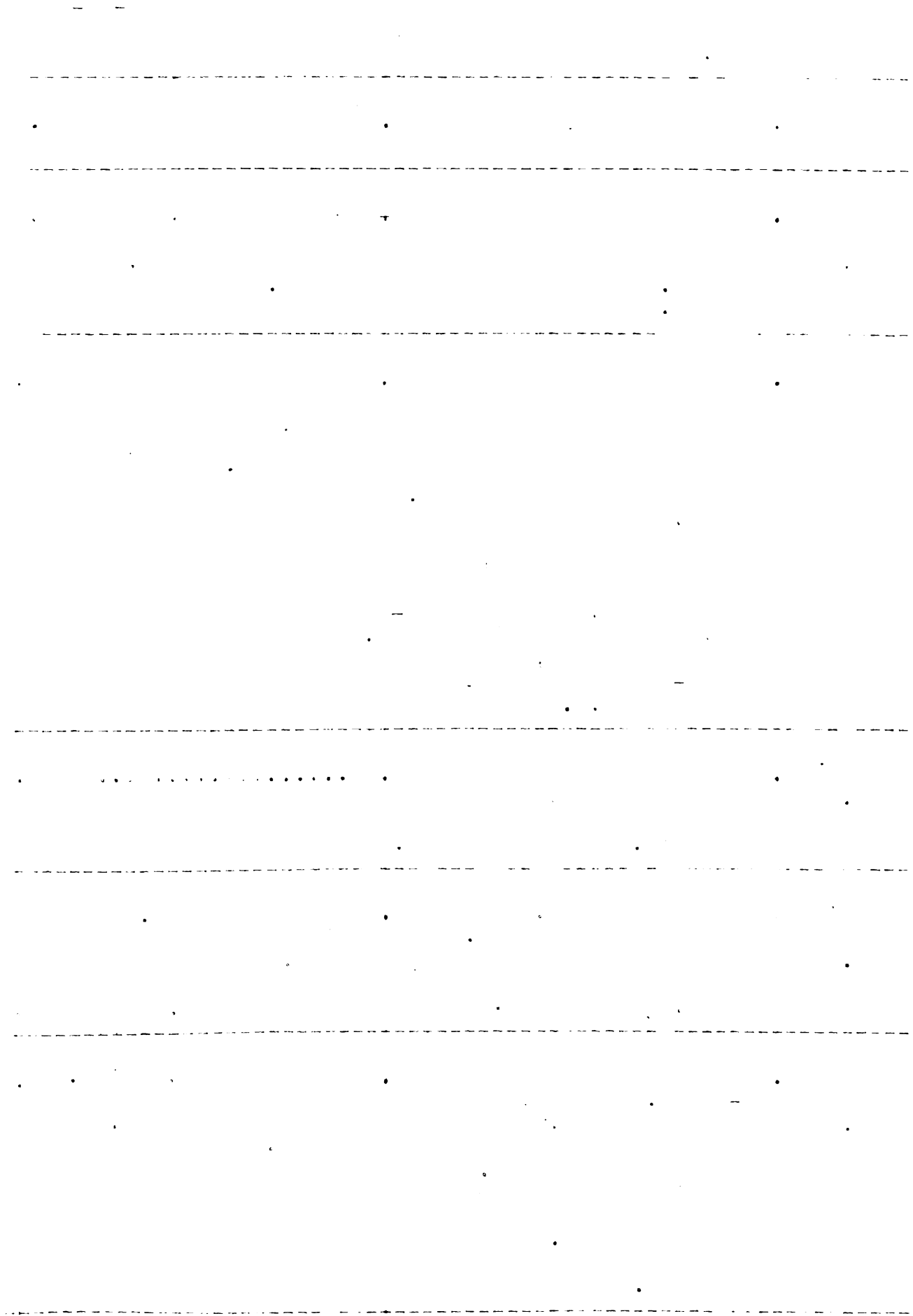
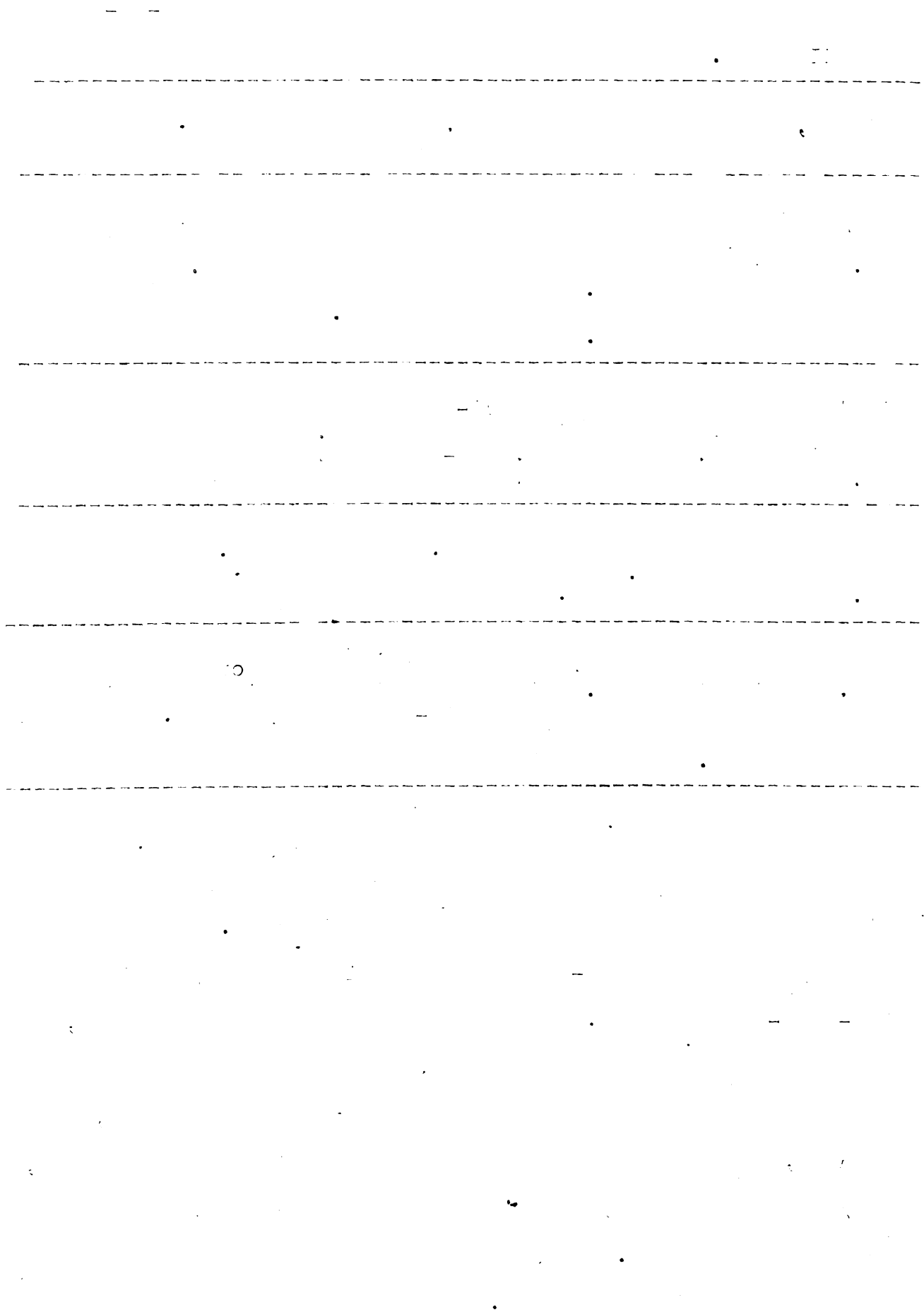


Table II Contd.

Medium	Parent	Variant.
Czapek Dextrose Agar.	Surface growth deep olive grey, variations white, dark olive grey or light vinaceous fawn. The medium colored dark heliotrope or vinaceous grey.	Growth abundant, white or vinaceous grey and light vinaceous fawn. In the medium a Dark Heliotrope grey.
Shive's Best Synthetic Agar.	Surface growth buffy citrine, dull citrine at places. White tufts. Substratumummy brown.	White and honey yellow tints. Substratum Russian Brown.
Lima Bean Agar.	Surface growth mouse grey. White tufts. Substratum olivaceous black.	Growth white. Substratum Benzo brown.
Tomato stems.	Abundant growth, brownish olive in color. Slightly purple or white hyphae mixed with the brownish olive growth.	Abundant growth very pale light pinkish lilac in color. No spores.

A study of the above table shows that both parent and variant varied considerably in color when grown on different media but the variations were along parallel lines. The parent form varied along olive-tawny shades and the variant in white-pink-lilac shades. The variant was constant throughout, while the parent continued sporting, giving off the variant form at different times on the following media: Corn Meal Flask, Oat Meal Agar, Prune Juice Agar, Rice Flask, Leonian Synthetic Agar, Czapek Synthetic Agar, Shive's Best Synthetic Agar, Lima Bean Agar and Tomato Stems. No spores were produced by the variant at any time on all these media. On proteinaceous media like



Lima Bean Agar and Nutrient Agar the surface color is rather gray while the color diffusing through the medium is nearly black.

The variations in shade of color on the different media were not constant but were lost on transferring to another medium and the color characteristic on that second medium assumed. Both the parent and the variant were grown on a set each of Oat Meal Agar, Lima Bean Agar and Nutrient Agar slants, the surface color was noted and then transferred to Nutrient agar. While the color on the three media referred to was variable, there was no difference at all when the transfers on Nutrient agar were compared. Table III illustrates this point.

Table III. Variations in shade of color are not constant but characteristic of the medium: Tests with Cladosporium fulvum parent strain and variant strain.

Medium	Surface color	Color on being transferred on Nutrient Broth agar.
<u>Parent</u>		
Oat Meal Agar.	Cinnamon Brown	Citrine Drab or Hathi Grey at places.
Lima Bean Agar.	Light vinaceous lilac	
Nutrient Agar.	Taupe Brown	
<u>Variant.</u>		
Oat Meal Agar.	White & Light brown- ish drab.	White or pallid purplish grey.
Lima Bean Agar.	" " " & pale vinaceous fawn.	
Nutrient Agar.	" " " & Pallid vinac- eous drab.	

NUTRITIONAL STUDIES.

The nutrition of fungi has been studied by many investigators, and while various plant decoctions are used as culture media, the importance of a knowledge of the exact composition of a culture medium has also been recognized and for physiological work a number of synthetic solutions containing those chemicals which are considered indispensable for proper growth have been devised. Solutions, however, that have been developed for a particular set of conditions and for a particular fungus may not at all meet the requirements of another fungus. On the contrary, the work of Coons in 1916 (24), Young and Bennett in 1922 (22) and probably of others shows that the limits of best growth and fructification vary and that a medium known to be best for a particular fungus is not suited to some other. Moreover, taking into account the different but definite response to environmental conditions as shown by Stevens and Hall (21) and Coons (24), it is easily seen that food relations must play an important role in variations. It has been observed that the variations obtained were in the nature of a loss of sporulation with a loss of the color and that these two characters were linked together in their persistence. In order to determine something of the influence of the nutrient salts and sugar on the color production and fruiting of the fungi under investigation the following set of experiments was conducted.

Coons synthetic solution (24) was devised with an aim at inducing sporulation. It was thought that increasing or decreasing the proportions of the salts above and below the optimum concentrations for this medium, a set of conditions might be

obtained favorable for the study of color production by the different fungi. The results of such experiments might further show whether it was the abundance or lack of a particular element that induced the permanent variations.

Coons' solution is made of the following ingredients:
Per 100 cc. of Medium:

M/5 Maltose	5 cc.
M/5 Asparagin	1 cc.
M/5 KH_2PO_4	5 cc.
M/5 MgSO_4	1 cc.

By arranging the number of cc. of M/5 of each of the above nutrients in a series according to the method used by the Committee on Salt Requirements of Plants National Research Council, for work in the study of nutrition of higher plants, and adding 2% agar and enough water to make up 100 cc. of solution, a number of culture media were obtained each varying by increments of 1 cc. of M/5 from the preceding and following culture. The amount of MgSO_4 was constant in all the cultures and while maltose and KH_2PO_4 were varied both above and below the amount given in the regular formula, Asparagin was only increased. A four figure number was given to each solution in which the first number stands for the number of cc. of M/5 Maltose, in 100 cc. of medium, the second for the number of cc. of M/5 KH_2PO_4 , the third for the number of cc. of M/5 Asparagin and the fourth for the number of cc. of M/5 MgSO_4 . For example, the number for the regular formula of Coons Medium is 5511 meaning 5 cc. M/5 Maltose, 5cc. M/5 KH_2PO_4 , 1 cc. M/5 Asparagin, and 1 cc. M/5 MgSO_4 per 100 cc. of medium.

The ingredients were mixed in the correct proportions and the necessary amount of water containing the agar in solution was added to make 100 cc. of medium. The medium was tubed, steam-



sterilized on 3 successive days and then slanted. The agar was added in such an amount as to give 2% in each medium.

It must be added here that these experiments were intended as preliminary, since it may be easily observed that the reaction of the different media differed as did also the osmotic pressures. This investigation, however, had to be discontinued at the end of August, 1922, and the results of this part of the work are only offered as a possible starting point for some succeeding investigator. It was originally planned to vary also the amount of H_2SO_4 , keeping the sugar constant but time was not available for this experiment either. Fifteen different media were prepared as described above and arranged in the following triangular form:

Maltose				
1st figure				
7311				
**				
6321		6411		
**		**		
5331		5421	5511	
**		**	**	
4341	4431	4521	4611	
**	**	**	**	
3351	3441	3531	3621	3711
**	**	**	**	**
Asparagin			Potassium Acid	
3rd Figure.			Phosphate.	
			2nd Figure.	

Since this experiment was intended to test the possibility of inducing variations in color according to food supply, it was thought best to use solid media where the color could be better observed and compared. It was therefore, impossible to determine amounts of growth, although in one case a set of

Liquid Media was prepared in 60 cc, Erlenmeyer Flasks containing each 20 cc. of the particular medium, and Colletotricum lindemuthianum Exp. Sta. Strain II grown on it. This experiment will be described in detail further on. Septoria apii, Colletotrichum lindemuthianum Exp. Sta. Str. I & II and Cladosporium fulvum both parent and variant were grown each in duplicate on this series of solid media. The following is an account of the behaviour of each fungus on this set of media.

SEPTORIA APII. There was no noticeable variation in color of this fungus grown on a set of media as described above. The fungus was always black with an abundance of pycnidia. There is perhaps a difference in the amount of growth in the various media and this can be easily observed in the picture of the set taken seventy days after inoculation. (Plate IV.) The darkest color is towards the top of the triangle where sugar was present in the greatest proportion, 5421 and 6411 being the cultures making about the best growth. It may also be seen that a slight white tuft appeared on 3531 and 3621 of the base line of cultures. The decrease in the proportion of phosphorus seems to have influenced growth much more effectively than the decrease in the proportion of nitrogen in the form of asparagin, sugar and magnesium being constant. Potassium varied in the same way as phosphorus since KH_2PO_4 was the salt used.

COLLETOTRICHUM LINDMUTHIANUM Exp. Sta. Strains I & II. grown each on a set of media as described above did not show many differences. The media were in both cases inoculated with spores and the cultures were allowed to grow under natural conditions, side by side in the laboratory. The pictures (Plate

V & VI) were taken seventy five days after inoculation and give a good idea of the exact appearance. Acervuli with the characteristic salmon pink color developed normally on all of them, independently of the black color, as foreexample on the excess Nitrogen side the black color developed very much later, the acervuli appearing first. The same was the case with the Lecnian medium which, as mentioned previously is Coons medium with five times the amount of nitrogen. The most growth occurred on the Sugar-Phosphorus side with decreasing intensity of color as the amount of sugar decreased and Phosphorus increased. Not a great number of white tufts were seen. Several white patches were observed in culture 5511 (Normal Coons Medium) of strain I but on being transferred to Cat Meal Agar, Corn Meal Agar and Corn Meal Flax they invariably grew black.

The variations in the amount of growth may be better studied in the liquid culture experiment to be described later on.

CLADOSPORIUM FULVUM was more responsive to such treatment than any of the fungi under study. Both the brown and the white form were grown each on a set of media as described above and the results were very interesting; especially the variations in color. This fungus makes a slow and moderately abundant growth and it seems that there was enough food for its development in all the cultures, since there was very little variation in the amount of growth. The only variations were in the color of the aerial part of the colony, the color or absence of color diffusing through the substratum and the difference in color of the submerged growth. Nevertheless the growth on media 5421 and 3441 seemed to be a little more abundant than on the others.

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100-100000

Regarding the color diffusing through certain media it was found that the color on Oat Meal Agar and Corn Meal Agar was mar/oon and a similar pigment appeared on media 4341 (Van Dyke Red) 3441 (Faint Van Dyke Red). Both these media are at the Nitrogen corner of the triangle. In the rest of the media the submerged growth was of a brownish olive color tinged more or less with ochraceous tawny especially toward the sugar side.

The color of the aerial part of the colony varied considerably according to the composition of the medium. In general it was brownish towards the sugar column, pinkish towards the nitrogen side and olive towards the Potassium-Phosphorus side. The intermediate cultures merged one into the other. Table IV gives the colors observed on the different media. The first line in each case is the surface color, the second the color of the submerged growth, the third variations of surface color the fourth the color diffused through the medium.

and also (TABLE IV on page 38.)

The number after each color refers to the plate in Ridgway's color standards.(63)

White mycelium appeared in media Nos 5331, 5421, 4431, on the sugar-nitrogen side of the triangle.

The white strain of *Cladosporium fulvum* was also grown on a similar series of media. Table V gives the colors observed on the different media. The growth was uniform in size of colony, 1/2 to 1 cm, in diameter, except on 3351 where the colonies were from 1-3 mm. in diam. The white character of the variation was preserved throughout unchanged, except that a yellowish tinge developed towards the sugar side, purplish

TABLE IV.

Color Variation of *Cladosporium fulvum* parent strain grown on Coons Medium varied in composition according to the triangular system.

7311

Sugar.

1. Ochraceous Tawny 45
2. Brownish Olive 30

6321

1. Grayish olive 46

6411

1. Honey Yellow 30
2. Brownish Olive 30
3. Pale Olive Buff 40
4. Light Brown Olive

5331

1. Saccardo's olive 16
2. Brownish Olive 30
3. White.

5421

1. Isabella Color 30
2. Brownish Olive 30
3. White

5511

1. Honey Yellow 30
2. Brownish Olive 30

4331

1. Pale Grayish Vinaceous 39
2. Brownish olive 30
3. Deep Olive Grey
4. Van Dyke Red 13

4431

1. Buffy Brown 40
2. Dark Olive 40
3. White

4521

1. Grayish olive 46
2. Brownish " 30

4611

1. Buffy Olive 30
2. Brownish Yellow 30

3351

1. Hydrangia Pink 27
2. Brownish Olive 30

3441

1. Pale Pinkish Buff 29
2. Brownish Olive 30
3.
4. Faint Van Dyke Red 13

3531

1. Grayish olive 46
2. Brownish " 30

3621

1. Dark Olive Buff 40
2. Brownish Olive 30

3711

1. Light Brownish Olive 30
2. Brownish Olive 30

aragin

Phosphate

1. Surface color.
2. Color of the submerged growth.
3. Variation on the surface.
4. Pigment diffusing through the medium.

TABLE V.

Color Variation of the Variant of *Cladosporium fulvum* grown on Coons Medium varying in composition according to the triangular system.

Maltose

7311

1. Cream Color	16
2. Buckthorne Brown	15
3.	
4. Brownish olive	30

6321

1. Buffy brown	40
2. White	
3. Fawn Color	40
4.	

6411

1. Tiller Buff	40
2.	
3.	
4. Dusky Olive Green	40

5331

1. White	
2. Cameo Pink	26
3. Deep Vinaceous	27
4.	

5421

1. Seashell Pink	14
2. White	
3. Worm Sepia	29
4. Moss Yellow	3

5511

1. White	
2. Pale Olive Buff	40
3. Brownish Olive	30
4.	

4341

1. White	
2. Pale lilac	37
3. Moss Violet	28
4. Ochre Red	27

4431

1. White	
2.	
3. Veronica Purple	38
4.	

4521

1. White	
2.	
3. Cinnamon brown	
4.	

4611

1. White	
2.	
3. Light Br. Olive	30
4. Brownish Olive	30

3351

1. White	
2.	
3. Vinous Purple	38
4. Olive	40

3441

1. White	
2. Pale lilac	37
3. Vinaceous Purple	38
4.	

3531

1. White	
2. Pale lilac	37
3. Moss Violet	38
4.	

3621

1. White	
2. Pale Shell Pink	28
3. Corinthian Purple	38
4.	

3711

1. White	
2. Cream color	16
3. Br. Olive	30
4. " "	30

argin

1. Surface color
2. Tinge
3. Color of colony seen through the slant.
4. Color of submerged growth.

Phosphate.



towards the nitrogen side and yellow-pink towards the Potassium-phosphorus side. On account of the purple tinge the white was more striking and clear on the **nitrogen** side.

Table V on page 39.

LIQUID MEDIA. Only Colletotrichum linderothianum Strain II was grown on liquid media. Twenty cc. of each medium in the set was placed in a 60 cc. Erlenmeyer flask and inoculated with .1 cc. of a spore suspension of the fungus. The cultures in duplicate were grown at room temperature on clinostat, ^(to insure even lighting) for 76 days. At the end of this period careful notes were taken, the flasks were photographed (Plate VII) and their contents filtered on weighed filter paper. The mycolial mat and the filter paper were dried to a constant temperature and reweighed. Table VI records the weights of the duplicates in each medium, their average and descriptions of their growth and color.

Table VI.

Means of growth of Colletotrichum linderothianum grown on liquid Coons medium varied according to the triangular system together with a macroscopic description of the cultures.

Medium	1st Ser. mgs.	2nd Ser. mgs.	Average mgs.	Description
7311	17.30	23.00	20.10	Abundant growth, submerged mycelium dirty white, a ring of Natal brown adhering to the glass on the surface of the medium.
6321	9.9	9.0	9.4	Wide blackish brown ring around the edge, with black small sclerotia like formations scattered through it. Submerged mycelium white.

Blank lined paper with horizontal ruling lines and scattered dots.

Table VI contd.

Medium	1st Ser. mgs.	2nd Ser. mgs.	Average mgs.	Description.
6411	15.5	25.0	20.2	No ring, no sclerotia, white mycelium, Royal brown patches on the surface.
5331	.8	13.5	6.3	Abundant white mycelium. Concentrically arranged sclerotia on the surface. Narrow, black ring discontinuous.
5421	4.7	7.1	5.9	Mycelium brownish. Concentrically arranged sclerotia on surface. Discontinuous black ring.
5511	10.1	10.0	10.0	Mycelium pale brown. Ring indefinite.
4341	4.7	12.8	8.7	Mycelium pure white. discontinuous, loose black ring.
4431	4.0	6.3	5.1	Mycelium nearly white. Wide, compact, blackish brown, discontin. ring.
4521	5.9	4.2	5.0	Mycel. dirty white. Black, compact, 1 cm. in diam. patches on the surface.
4611	2.7	6.3	4.5	Mycel. brownish. Indefinite ring brown in color with sclerotia.

• **Prevalence** – the proportion of a population that has a disease at a particular point in time

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Journal of Management Education 30(6)p.789-804
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• *Journal of the American Medical Association*, 2000; 284: 2561-2566

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• *Chlorophyll a* (Chl a) is the primary photosynthetic pigment in all photosynthetic organisms. It is a green pigment that absorbs light energy in the blue and red regions of the visible spectrum. Chl a is found in the thylakoid membranes of chloroplasts in plants and in the plasma membrane of cyanobacteria and algae. It plays a central role in the light reactions of photosynthesis, where it captures light energy and transfers it to other components of the photosynthetic apparatus.

— **1998** **1999** **2000** **2001** **2002** **2003** **2004** **2005** **2006** **2007** **2008** **2009** **2010** **2011** **2012** **2013** **2014** **2015** **2016** **2017** **2018** **2019** **2020** **2021** **2022** **2023** **2024** **2025** **2026** **2027** **2028** **2029** **2030** **2031** **2032** **2033** **2034** **2035** **2036** **2037** **2038** **2039** **2040** **2041** **2042** **2043** **2044** **2045** **2046** **2047** **2048** **2049** **2050** **2051** **2052** **2053** **2054** **2055** **2056** **2057** **2058** **2059** **2060** **2061** **2062** **2063** **2064** **2065** **2066** **2067** **2068** **2069** **2070** **2071** **2072** **2073** **2074** **2075** **2076** **2077** **2078** **2079** **2080** **2081** **2082** **2083** **2084** **2085** **2086** **2087** **2088** **2089** **2090** **2091** **2092** **2093** **2094** **2095** **2096** **2097** **2098** **2099** **2100** **2101** **2102** **2103** **2104** **2105** **2106** **2107** **2108** **2109** **2110** **2111** **2112** **2113** **2114** **2115** **2116** **2117** **2118** **2119** **2120** **2121** **2122** **2123** **2124** **2125** **2126** **2127** **2128** **2129** **2130** **2131** **2132** **2133** **2134** **2135** **2136** **2137** **2138** **2139** **2140** **2141** **2142** **2143** **2144** **2145** **2146** **2147** **2148** **2149** **2150** **2151** **2152** **2153** **2154** **2155** **2156** **2157** **2158** **2159** **2160** **2161** **2162** **2163** **2164** **2165** **2166** **2167** **2168** **2169** **2170** **2171** **2172** **2173** **2174** **2175** **2176** **2177** **2178** **2179** **2180** **2181** **2182** **2183** **2184** **2185** **2186** **2187** **2188** **2189** **2190** **2191** **2192** **2193** **2194** **2195** **2196** **2197** **2198** **2199** **2200** **2201** **2202** **2203** **2204** **2205** **2206** **2207** **2208** **2209** **2210** **2211** **2212** **2213** **2214** **2215** **2216** **2217** **2218** **2219** **2220** **2221** **2222** **2223** **2224** **2225** **2226** **2227** **2228** **2229** **2230** **2231** **2232** **2233** **2234** **2235** **2236** **2237** **2238** **2239** **2240** **2241** **2242** **2243** **2244** **2245** **2246** **2247** **2248** **2249** **2250** **2251** **2252** **2253** **2254** **2255** **2256** **2257** **2258** **2259** **2260** **2261** **2262** **2263** **2264** **2265** **2266** **2267** **2268** **2269** **2270** **2271** **2272** **2273** **2274** **2275** **2276** **2277** **2278** **2279** **2280** **2281** **2282** **2283** **2284** **2285** **2286** **2287** **2288** **2289** **2290** **2291** **2292** **2293** **2294** **2295** **2296** **2297** **2298** **2299** **2300** **2301** **2302** **2303** **2304** **2305** **2306** **2307** **2308** **2309** **2310** **2311** **2312** **2313** **2314** **2315** **2316** **2317** **2318** **2319** **2320** **2321** **2322** **2323** **2324** **2325** **2326** **2327** **2328** **2329** **2330** **2331** **2332** **2333** **2334** **2335** **2336** **2337** **2338** **2339** **2340** **2341** **2342** **2343** **2344** **2345** **2346** **2347** **2348** **2349** **2350** **2351** **2352** **2353** **2354** **2355** **2356** **2357** **2358** **2359** **2360** **2361** **2362** **2363** **2364** **2365** **2366** **2367** **2368** **2369** **2370** **2371** **2372** **2373** **2374** **2375** **2376** **2377** **2378** **2379** **2380** **2381** **2382** **2383** **2384** **2385** **2386** **2387** **2388** **2389** **2390** **2391** **2392** **2393** **2394** **2395** **2396** **2397** **2398** **2399** **2400** **2401** **2402** **2403** **2404** **2405** **2406**

Table VI contd.

Medium	1st. Ser.	2nd Ser.	Average	Description.
3351	2.0	11.0	6.5	White mycel. Thin ring with sclerotia.
3441	6.4	2.1	4.2	Mycelium nearly white. Thin ring with sclerotia.
3531	2.0	4.8	3.3	Mycelium brown. Thick brownish ring.
3621	3.0	4.0	3.5	Mycel. brown, ring indefinite, sclerotia.
3711	...	2.9	2.9	Mycel. brown, ring indefinite, sclerotia.

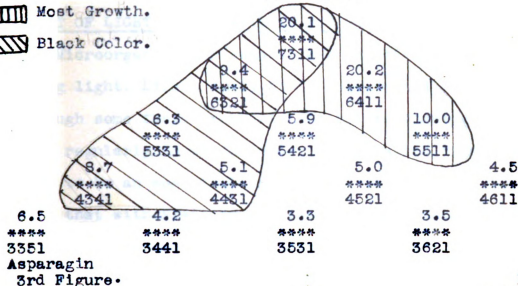
The mycelium from the different flasks was examined microscopically. The black or brown pigment was found in the mycelium. The bodies referred to in the table above as black sclerotia were tiny tangles of mycelium. Sporulation was found correlated with the color. Brown or white mycelium showed no spores. Transfers from several flasks showing white mycelium on Oat Meal Agar grew invariably black. This seems to indicate that the variations in color were not fixed but were due to nutritional factors, which prohibited the formation of spores especially towards the Potassium-phosphorus side of the triangle. Sporulation seems to be linked with the color character. ~~back~~ Whenever the aerial black ring or the black sclerotia were formed the mycelium was white and spores formed, but when no

DIAGRAMS illustrating the regions of greatest growth and development of pigment in cultures of Colletotrichum lindemuthianum grown on Coons Medium varying in composition according to the trangular system previously described.

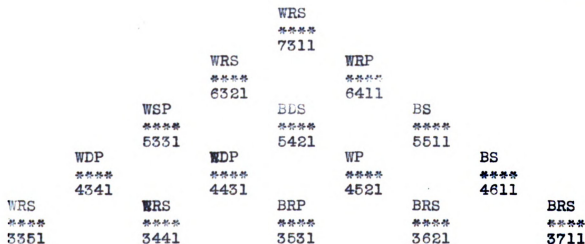
Maltose
1st figure.

Most Growth.

Black Color.



Potassium
Acid Phosphate.
2nd Figure.



Legend: W= White mycelium
B= Brown mycelium
R= Ring present
D= Ring discontinuous
S= Sclerotia
P= Patches of brown mycel. on surface.

such structures were produced the mycelium was brownish and no spores were found. Lack of proper nutrients more than any other factor caused these variations and sugar and nitrogen seem to be essential for both color and sporulation.

EFFECT OF LIGHT ON VARIABILITY AND COLOR.

Microorganisms in general are negatively phototropic under strong light. Direct sunlight is detrimental to most bacteria although some fungi are resistant to it. Fungi grow well and fruit regularly in diffused day light, which in many instances seems to be an essential factor for reproduction. Coons (24) found that with Plenodomus fuscomaculans light is a factor ~~at~~ directly concerned with pycnidium production, the fungus refusing to fruit when kept in the dark, irrespective of nutrition, aeration, substratum or strain. Makemson (54) found that light had a retarding effect upon the growing germ tubes of Cladosporium fulvum but the ultimate length was the same. Vegetative growth and sporulation was also more profuse when the fungus was grown in the dark. Stevens (83) observed slightly less zonation and less aerial mycelium in the dark than in the light, working with Helminthosporium sp. grown on Petri dishes. Smith and Swingle (81) report that diffused day light affected considerably the color of fusaria and Sherbakoff (78) found that diffused daylight intensifies the color of Fusaria, while intense light dulls it. He did not find light influencing other characters in fusaria. Older literature reviewed by Coons (24) and Harshberger (41) is just as contradictory. It seems that different fungi vary in their response to light of varying intensities some vegetating best in darkness and requiring the light stimulus to induce fruiting, others growing and fruiting best

in diffused daylight or in darkness.

In order to determine whether light had any effect on color changes and variations of the fungi under study, four sets of media as described under Nutritional Studies (p. 32) were inoculated with the white and brown strains of Cladosporium fulvum. A set of each strain was grown under a bell jar completely darkened to exclude light. A second set of test-tubes arranged and fastened on the walls of a battery jar, covered by a bell jar, was exposed to strong diffused light and gradually rotated to overcome differences in intensity. Air was circulated through both bell jars. The jars were not disturbed until the end of the experiment when the colors of the cultures in both sets on each medium were studied and pictures taken. (Plates 8-11.) In general light did not seem to effect the color or growth of the colony or the number of variations. A detailed description and comparison of the cultures on each medium in light and dark is given in tables VII, VIII, and IX.

Tables VII and VIII are diagrammatic, being further complemented by table IX and Plates VIII- XI. Slight variations in color as given in these tables were neither consistent nor in a definite direction, to be taken into consideration in concluding on the effect of the factor under study.

TABLE VII.

Color Variation of *Cladosporium fulvum*, parent form,
grown in light and dark on Coons medium varying in
composition according to the triangular system.

MALTOSE.

Light	7311	Dark
1. Ochraceous tawny	1. Buffly olive 30	
2. Brownish olive 20	2. Brownish olive 30	
3. 4 white colonies	3. 3 white col.	
4.		

Light	6321	Dark	Light	6411	Dark
1. Grayish Olive 46	Grayish Olive 46		1. Ochraceous tawny 15	Grayish Olive 46	
2.			2. Brownish Olive 30	Brownish Ol. 30	
3. White			3. 1 white colony	White 1 col.	
4.			4.	4.	

Light	5331	Dark	Light	5421	Dark	Light	5511	Dark
1. Van Dyke Br. 28	Van Dyke Brown 28		1. Bister 29	Saccardo's Umbrose		1. Brussels Br. 3	Brussels Brown 3	
2. Light Olive Gray 51	Light Olive Gray 51		2.	2.		2. Brownish Olive 30	Brownish Olive 30	
3.			3. White	3. White		3. White	3. White	
4.			4.	4.		4. Brown. Olive 30	4.	

Light	4341	Dark	Light	4431	Dark	Light	4521	Dark	Light	4611	Dark
1. Mummy Brown 15	Mummy Brown 15		1. Mummy Br. 15	Mummy Br. 15		1. Bister 29	Septa 29				
2.			2.	2.		2.	2.				
3. White	3. White		3. White	3. White		3. Grayish	3. White				
4. Carob Brown 14	Carob Brown 14		4. River Br. 14	4.		4.	4.				

Light	3351	Dark	Light	3441	Dark	Light	3531	Dark	Light	3621	Dark	Light	3711	Dark
1. Light Brown tawny 34	Light Br. tawny 34		1. Deep luscious (ash) 46	Olive Brown 30		1. Bister 28	1. Bister 29		1. Raw Umber 3	1. Raw Umber 3		1. Medal Bronze	1. Medal Bronze	
2. Clove Brown 40	Clove Br. 40		2.	2.		2.	2.		2.	2.		2.	2.	
3.	3.		3.	3. White		3.	3. White		3. White	3. White		3.	3.	
4.	4.		4. Mass violet 36	4. River Br. 14		4. Dark Mineral Red 23	4. Dark Mineral Red		4. River Br. 14	4.		4.	4.	

ASPARAGIN

1. Surface color.
2. Color of the submerged growth
3. Variation on the surface.
4. Pigment diffusing through the medium.

PHOSPHATE.

TABLE VIII.

Color Variation of *Gladosporium fulvum*, variant form,
grown in light and dark on Ooone Medium varying in
composition according to the triangular system.

-47-

MALTOSE

Light 7311 Dark

1. Cream Color 16	1. Naples Yellow 16
2.	2. White
3. Yellow Ochre 15	3. Yellow Ochre 15
4. Dusky Ol. Green 41	4. Dusky Olive Br. 41

Light 6321 Dark

1. Pale Ochraceous Salmon 15	1. Pale Ochraceous Salmon 15
2.	2.
3. Ochraceous Orange Ochraceous Buff 15	3. Ochraceous Orange Ochraceous Buff 15
4.	4. Dusky Ol. Green 41

Light 6411 Dark

1. Cinnamon Buff 24	1. Olive Buff 40
2.	2. White
3. Ochraceous Buff 24	3. Cinnamon Buff 24
4. Dusky Ol. Green 41	4. Dusky Ol. Green 41

Light 5331 Dark

1. White	1. White
2. Cameo Pink 26	2. Cameo Pink 26
3. Ochre Red 27	3. Ochre Red 27
4. Dusky Ol. Green 41	4. Dusky Ol. Gr. 41

Light 5421 Dark

1. White	1. Pinkish Buff 24
2. Pale Pinkish Cream 24	2. White
3. Vinaceous Tawny 24	3. Ochre Red 27
4. Sepia 24	4. Dark Olive 40

Light 5511 Dark

1. White	1. White
2.	2. Titheal Buff 40
3. Isabella Color 30	3. Isabella Color 30
4. Dark Olive 40	4. Dark Olive 40

Light 4341 Dark

1. White	1. White
2. Lilac 25	2. Lilac 37
3. Dark Purplish Purple 24	3. Neutral Red 38
4. Dark Indian Red 27	4. Light Steel Br. 39

Light 4431 Dark

1. White	1. White
2. Titheal Buff 40	2. Pale Lilac 37
3. Dark Vinaceous Br. 39	3. Deep Cerise Red 27
4. Hay's Br. 39	4. Helian Br. 13

Light 4521 Dark

1. White	1. White
2.	2.
3. Ferruginous 12	3. Prussian Red 37
4. Brownish Olive 30	4. Dark Olive 40

Light 4611 Dark

1. White	1. White
2.	2.
3. Light Steel Br. 39	3. Light Steel Br. 39
4. Brown Ol. 30	4. Brown Ol. 30

Broken

Light 3351 Dark

1. Pale Vinaceous Lilac 24	1. Pale Vinaceous Lilac 24
2.	2.
3. Dark Vinaceous Purple 24	3. Dark Vinaceous Purple 24
4. Dark Olive 40	4. Dark Olive 40

Light 3441 Dark

1. Faded Brown 39	1. White
2.	2. Pale Lilac 37
3. Mares Brown 39	3. Dull dusky Purple 24
4.	4.

Light 3531 Dark

1. White	1. White
2. Light Br. Vinaceous 39	2. Rosadine Pink 24
3. Silver Brown 15	3. Madder Br. 13
4.	4.

Light 3621 Dark

1. White	1. Light Buff 15
2. Light Vinaceous 39	2. Ochraceous Tawny 15
3. Prussian Red	3. Livers Brown
4.	4.

Light 3711 Dark

1. Light Buff 15	1. Light Buff 15
2.	2.
3. Ochraceous Tawny 15	3.
4. Prout's Brown 15	4.

ASPARAGIN

1. Surface color
2. Tinge
3. Color of colony seen through the
4. Color of submerged growth. [slant.

PHOSPHATE.

TABLE IX.

Comparison of two sets of cultures of Cladosporium fulvum Parent and Variant grown on Coons Medium varying in composition according to the triangular system, grown in light and dark.

Compo- sition of medium.	Description. Parent Strain.
7311.	L.(Light) and D. (Dark) are very much alike. Good growth. The color is the same, except that the buffy olive found in the center of a few colonies in L. colors most colonies in D. especially towards the base of the slant. Ochraceous tawny is seen at the margins of the buffy olive colonies of D.
6321	Remarkably uniform. No submerged growth.
6411	Quite different. D. is composed of a large colony with white aerial center, uniform in color. Abundant spores of same color. L. is composed of small colonies covering the entire slant, tawny olive in color with a few buffy olive and one white.
5331	Remarkably alike. Reddish brown and Van dyke Brown.
5421	Alike. L. more brown than olive green. Both show white variations.
5511	Exactly alike. L. has more white variations than D. No discoloration of the substratum.
4341	Alike. One large spreading colony in both.
4431	Similar in surface growth and amount of white variations. L. is made of one colony and shows color in substratum, D. is composed of small colonies and shows no color in substratum. At dry top of slant both show buffy olive growth.
4521	Almost alike. D. is a little more brown and shows more white variation which is not compact but loose fuzzy growth at the center of the colony. Submerged growth and the pigment through medium are of the same brown red color.
4611	Broken.
3351	Color alike in both. Colonies smaller in L.
3441	About the same. Vinaceous in L. rather brown in D. Color through medium more pronounced in L,



TABLE IX Continued.

- 3531 Surface color and type of growth similar. D. shows more pure white variation, a slight white fuzz on one colony in L. Color through substratum the same in both.
- 3621 Surface color the same in both. Colonies in D. smaller. No substratum color in D.
- 3711 Olive green. Mostly dried.

Variant Strain.

- 7311 D. and L. are almost alike in general appearance. Colonies large (1 cm. in diam.) fluffy, raised. D. is slightly darker and has a surface colony of pure white. The under side of the colony as seen through the agar is yellow ochre while the growth into the substratum and in the water of condensation (submerged) is olive green.
- 6321 D. and L. exactly alike. Mat like single colonies, white tinged with pink and ochre. Large guttation drops in the middle. No submerged growth in L.
- 6411 Uniform in shape of colony. L. is strongly tinged with ochre, more intense at some points than at others. The edges of D. are rather olive buff with the center white.
- 5331 Identical.
- 5421 Shape and form of colonies alike. Both show the buff pinkish tinge, L. being lighter, however.
- 5511 Small rounded colonies, white tinged with green. Some pink in D.
- 4341 Shape of colony the same. Colonies covered with small drops. Tinged with violet, more so in L.
- 4431 White colonies slightly tinged with purple. Under side of colonies of a mixture of purple, brown and olive.
- 4521 Fluffy colonies, with no drops on the surface. White slightly tinged with pink and ochre. Under side of colonies rather red in D., yellowish in L.
- 4611 White growth in both.
- 3351 Alike.
- 3441 D. white, L. brownish purple.
- 3531 White colonies, purplish tinge.
- 3621 Alike. Tinge slightly varying.
- 3711 Alike.

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EFFECT OF REACTION ON VARIABILITY AND COLOR.

The reaction of the substratum has recently been recognized as one of the most important environmental factors influencing the physiology of plants. It had been formerly ~~the~~ thought that adjusting the reaction of the medium used, by some sort of titration, to a point above or below the turning point of an indicator, usually phenolphthalein, was a satisfactory method of securing an optimum reaction for microorganisms. However, with the discovery and perfection of methods for measuring the active acidity in media, investigations were conducted which proved that the Hydrogen ion concentration should be classed with temperature and moisture as an important environmental factor. It was also found that besides influencing growth in general, the reaction has an effect on color production, fruiting, zonation etc.

Sherbakoff (78) in 1915 found that acidity induces the production of red color in those fusaria which make a grey-white growth on neutral media. Acidity also lowers the rate of growth and makes zonation prominent. Fungi are able to withstand a comparatively wide range of pH variation. Meacham (56) in 1918 found that Lenzite sapientaria, Fomes roseus, Merulius lachrymans and Coniophora cerebella would grow from pH 5-1.7 on the acid range. Webb (91) in 1919 studying the germination of spores of different fungi at different pH found that the range of spore germination with respect to the reaction of the medium is between pH 2-10 with maxima at pH 3-4 and 7. Johnson (46) in 1923 found that the reaction best suited for the growth of molds lies towards the acid range of the neutral



point. Goss (40) in 1924 found that Fusarium eumartii is capable of growing under a wide variation in the H-ion concentration on 2% dextrose potato agar. At optimum temperature no appreciable difference was seen in the growth on media varying in pH from 4.5 to 8.5. Hopkins in 1922 (44) found that by adding 3 drops of 50% lactic acid to 20 cc. agar, a strain of Colletotrichum lindemuthianum which produced few spores in neutral potato agar, sporulated freely.

In order to determine the effect of variation in the pH of the medium on which the fungi under investigation were grown, the following experiments were undertaken. Since the variations observed were in two directions, viz: a lack or modification of pigment and the suppression of sporulation, it was thought best to use two kinds of media; one favoring fruiting and the other favoring vegetative growth. Coons medium (24) was found by its originator to actually favor fruiting and the behaviour of Sphaeropsis mal/orum in the present investigation also indicated the tendency of this ~~medium~~ medium to induce spore formation. Richards medium E (67) has been frequently used by many investigators as a general synthetic medium with good results. The modification by Karrer and Webb (49) was used in order to avoid the precipitation of the phosphate in the alkaline members of the series. Liquid media were used in this experiment and the filter paper cone preparation-dish method was adopted as reported by Coons (24) and recently by Bonar (12). This method consists in growing the fungus on a filter paper cone placed in a small deep petri dish (preparation dish) with 10 cc. of medium. Schleicher & Schull No 595 7 cm. in diam. paper was used. The deep petri

dishes about 5 cm. in diam. and 3 cm. deep, were again protected by placing them in large moist chambers or under bell-jars. Under such conditions the fungus grows on a solid substratum of pure cellulose kept at constant saturation. Both the white and the parent strains of Cladosporium fulvum and Colletotrichum lindemuthianum Exp. Sta. Str. II were grown in duplicate series of 10 different H-ion concentrations varying from pH 2 to 8.4. Besides the paper cone cultures, the fungi were grown in an exactly similar series of media in test tubes with the proper indicator added to the medium. The purpose of this experiment was to determine as far as possible the shifting of the pH by the growth of the fungus.

Previous to starting the experiment the titration curves of both media to be used were determined. Karrer and Webb (49) report a titration curve for Richard's medium E modified, but the present writer was unable to duplicate their findings. The pH of Richard's solution is given by them as 4.6 while with the particular chemicals, apparatus, etc. with which the writer worked, it was found to be 5.0. This finding necessitated the retitration of the medium. Table X gives the number of cc., the normality of acid and alkali, and the number of cc. of water to be added to 25 cc. of double strength medium to make 50 cc. of medium of a desired pH and of the proper concentration in nutrients. It was found that by using weaker solutions of acid and alkali than those recommended by Karrer and Webb (49), a smoother curve could be obtained. Fig. 1 gives the titration curves and Table XI the titration values.

The preparation dishes with the filter paper cones in place

TABLE X.

Co. of Acid or Alkali and Water to be added to 25 cc. of double strength medium to obtain a series of media of varying pH and proper concentration of nutrients.

pH	cc. HCl	Normality	cc. NaOH	Normality	cc. H ₂ O	Volume
RICHARDS						
8.0	11	N/20	**	**	14	50
2.8	7	N/50	**	**	18	50
3.7	1.5	" "	**	**	23.5	50
4.8	.25	" "	**	**	24.75	50
5.0	**	**	**	**	25	50
5.8	**	**	.5	N/20	24.5	50
6.4	**	**	1.0	" "	24	50
7.0	**	**	2.5	" "	22.5	50
7.6	**	**	4.0	" "	21	50
8.4	**	**	6.0	" "	19	50
COONS						
2.4	20	N/50	**	**	5	50
2.8	8	" "	**	**	17	50
3.2	4	" "	**	**	21	50
3.8	1.5	" "	**	**	23.5	50
4.2	.5	" "	**	**	24.5	50
5.0	**	**	**	**	25	50
5.6	**	**	.5	N/20	24.5	50
6.4	**	**	2.5	" "	22.5	50
7.0	**	**	5.5	" "	19.5	50
7.6	**	**	7.5	" "	17.5	50
8.4	**	**	9.5	" "	15.5	50



TABLE X.

Co. of Acid or Alkali and Water to be added to 25 cc. of double strength medium to obtain a series of media of varying pH and proper concentration of nutrients.

pH	cc. HCl	Normality	cc. NaOH	Normality	cc. H ₂ O	Volume
RICHARDS						
2.0	11	N/20	**	**	14	50
2.8	7	N/50	**	**	18	50
3.7	1.5	" "	**	**	23.5	50
4.8	.25	" "	**	**	24.75	50
5.0	**	**	**	**	25	50
5.8	**	**	.5	N/20	24.5	50
6.4	**	**	1.0	" "	24	50
7.0	**	**	2.5	" "	22.5	50
7.6	**	**	4.0	" "	21	50
8.4	**	**	6.0	" "	19	50
COONS						
2.4	20	N/50	**	**	5	50
2.8	8	" "	**	**	17	50
3.2	4	" "	**	**	21	50
3.8	1.5	" "	**	**	23.5	50
4.2	.5	" "	**	**	24.5	50
5.0	**	**	**	**	25	50
5.6	**	**	.5	N/20	24.5	50
6.4	**	**	2.5	" "	22.5	50
7.0	**	**	5.5	" "	19.5	50
7.6	**	**	7.5	" "	17.5	50
8.4	**	**	9.5	" "	15.5	50

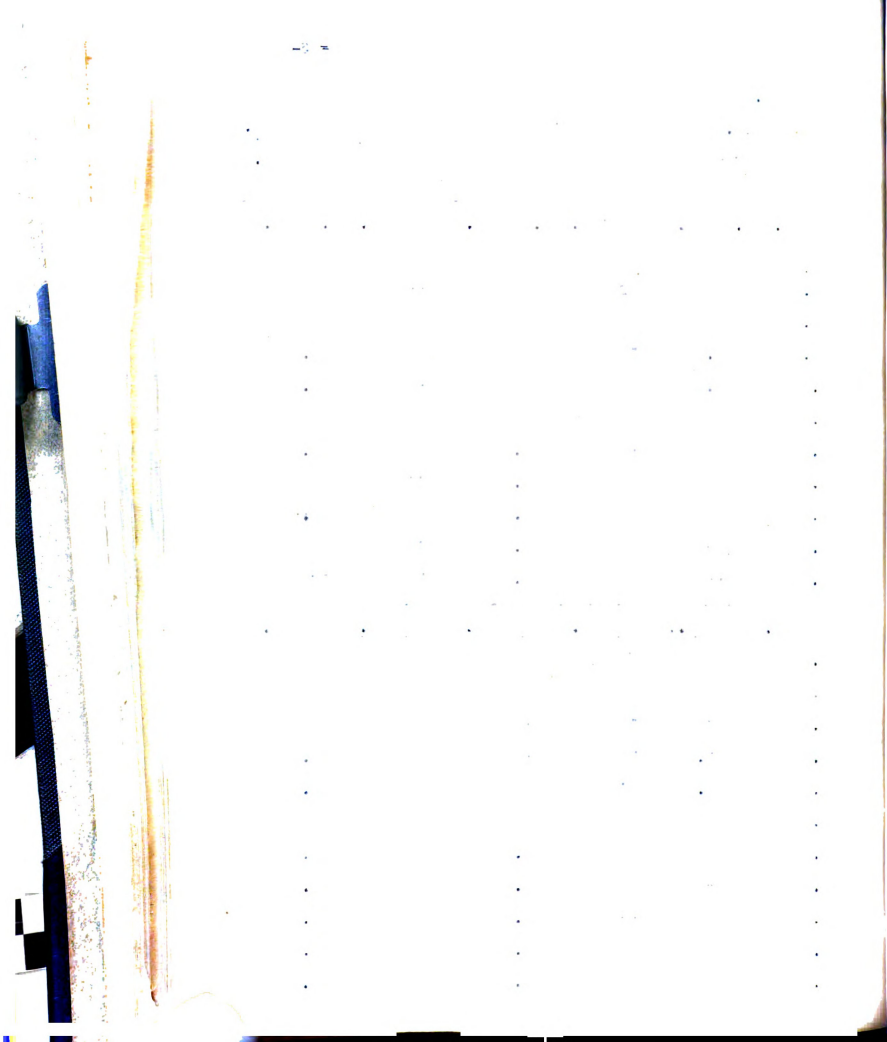
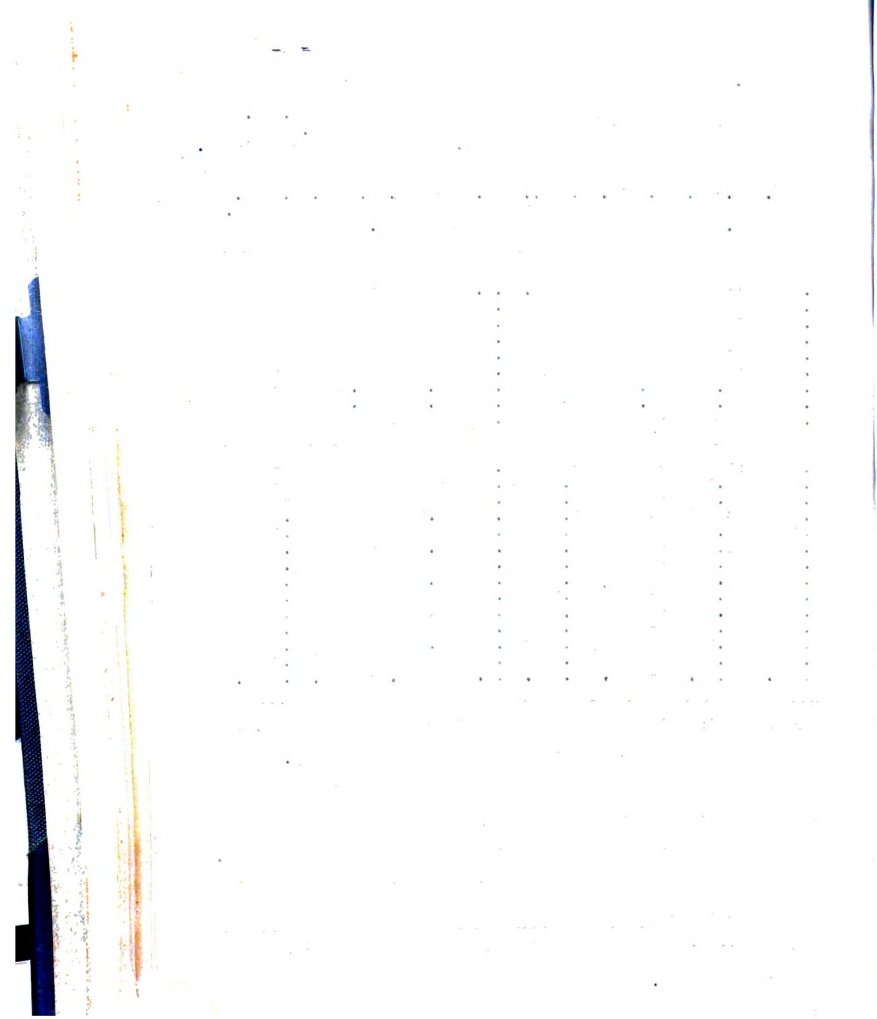


TABLE XI.

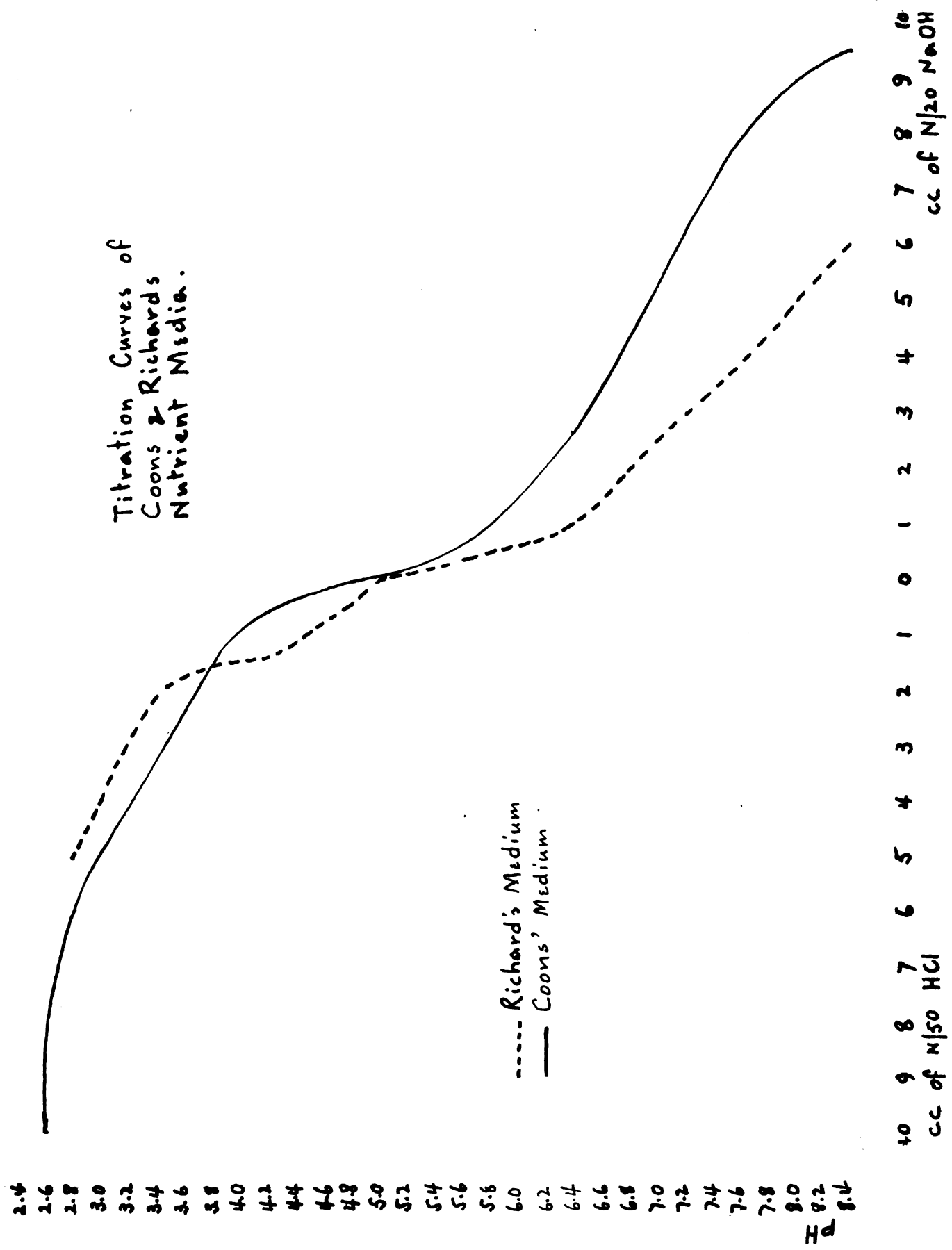
TITRATION DATA FOR COONS AND RICHARDS SOLUTIONS. cc. of Acid or Alkali and Water to be added to 25 cc. of Double Strength medium to give 50 cc. of Medium of a certain pH.

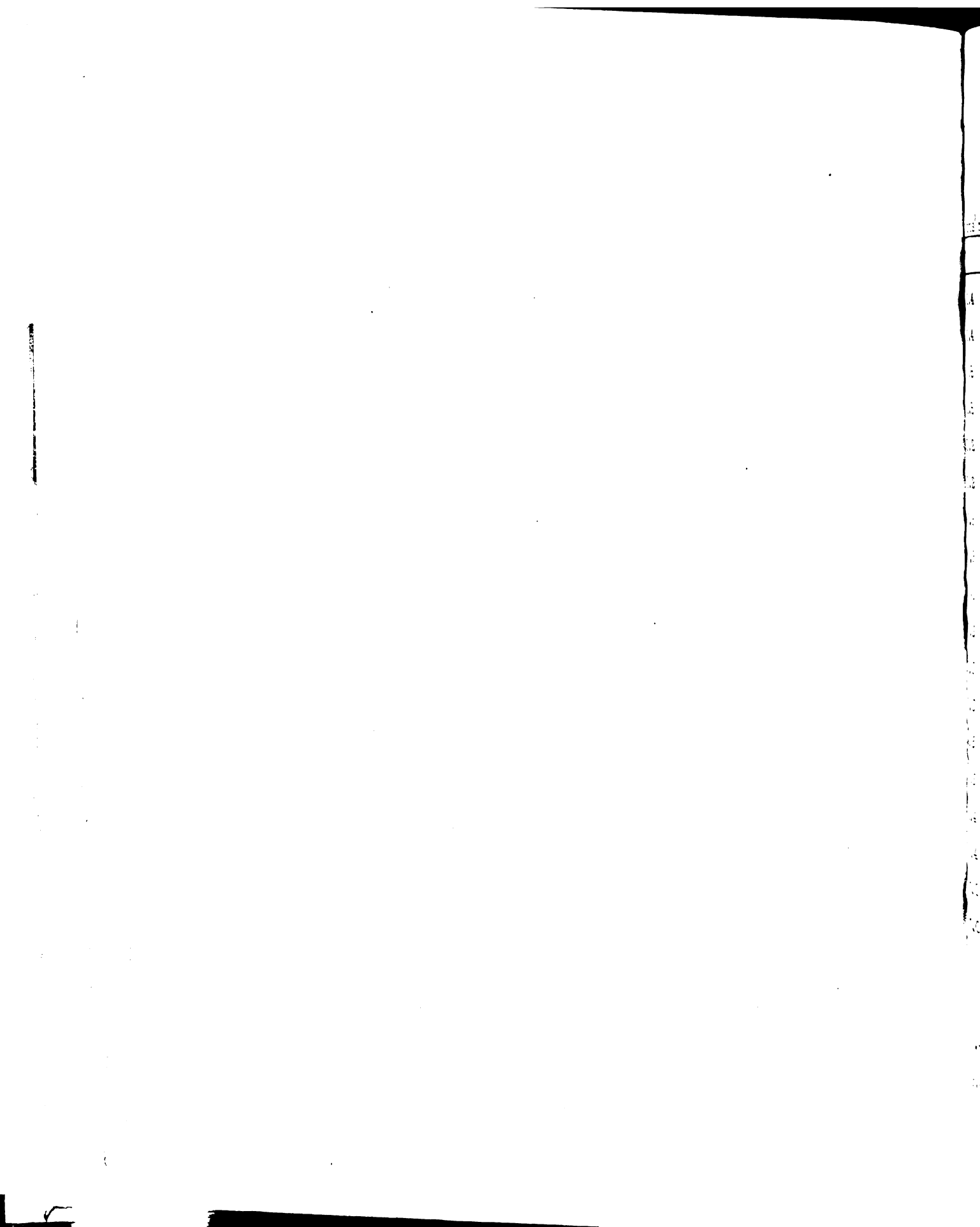
pH	cc. H ₂ O	cc. Acid N/50HCl	cc. Alk. N/20NaOH	pH	cc H ₂ O	cc. Acid N/50HCl	cc. Alk. N/20NaOH.
C O O N S.				R I C H A R D S.			
2.2	**	25	**	2.2	5	20	**
2.6	15	10	**	2.6	**	**	**
2.8	19	6	**	2.8	20	5	**
3.0	20	5	**	3.0	21	4	**
3.2	21	4	**	3.2	22	3	**
3.4	22	3	**	3.4	23	2	**
3.8	23.5	1.5	**	3.8	23.5	1.5	**
4.2	24.5	.5	**	4.2	24.5	.5	**
5.0	25	**	**	5.0	25	**	**
5.0	25	**	**	5.0	25	**	**
5.4	24.75	**	.25	5.4	**	**	**
5.6	24.5	**	.50	5.6	**	**	**
5.8	24	**	1.0	5.8	24.5	**	.5
6.4	22.5	**	2.5	6.4	24	**	1.0
6.6	21.5	**	3.5	6.6	23.5	**	1.5
6.8	20.5	**	4.5	6.8	23	**	2.0
7.0	19.50	**	5.5	7.0	22.5	**	2.5
7.2	18.5	**	6.5	7.2	22	**	3.0
7.4	17.5	**	7.5	7.4	21.5	**	3.5
7.6	17.0	**	8.0	7.6	21	**	4.0
7.8	16.5	**	8.5	7.8	20.5	**	4.5
8.0	16.0	**	9.0	8.0	20	**	5.0
8.4	15.5	**	9.5	8.4	19	**	6.0

were sterilized in the dry air sterilizer while the medium, the water and the acid or alkali were autoclaved separately. The proper amounts were mixed in a thoroughly steamed inoculation chamber and pipetted into the tubes and the preparation dishes by means of sterile pipettes provided with cotton filter plugs. A suspension of spores was used as inoculum in the case of the parent strains of Colletotrichum lindemuthianum and Cladosporium fulvum while in the case of the variants of both fungi a bit of mycelium was used.



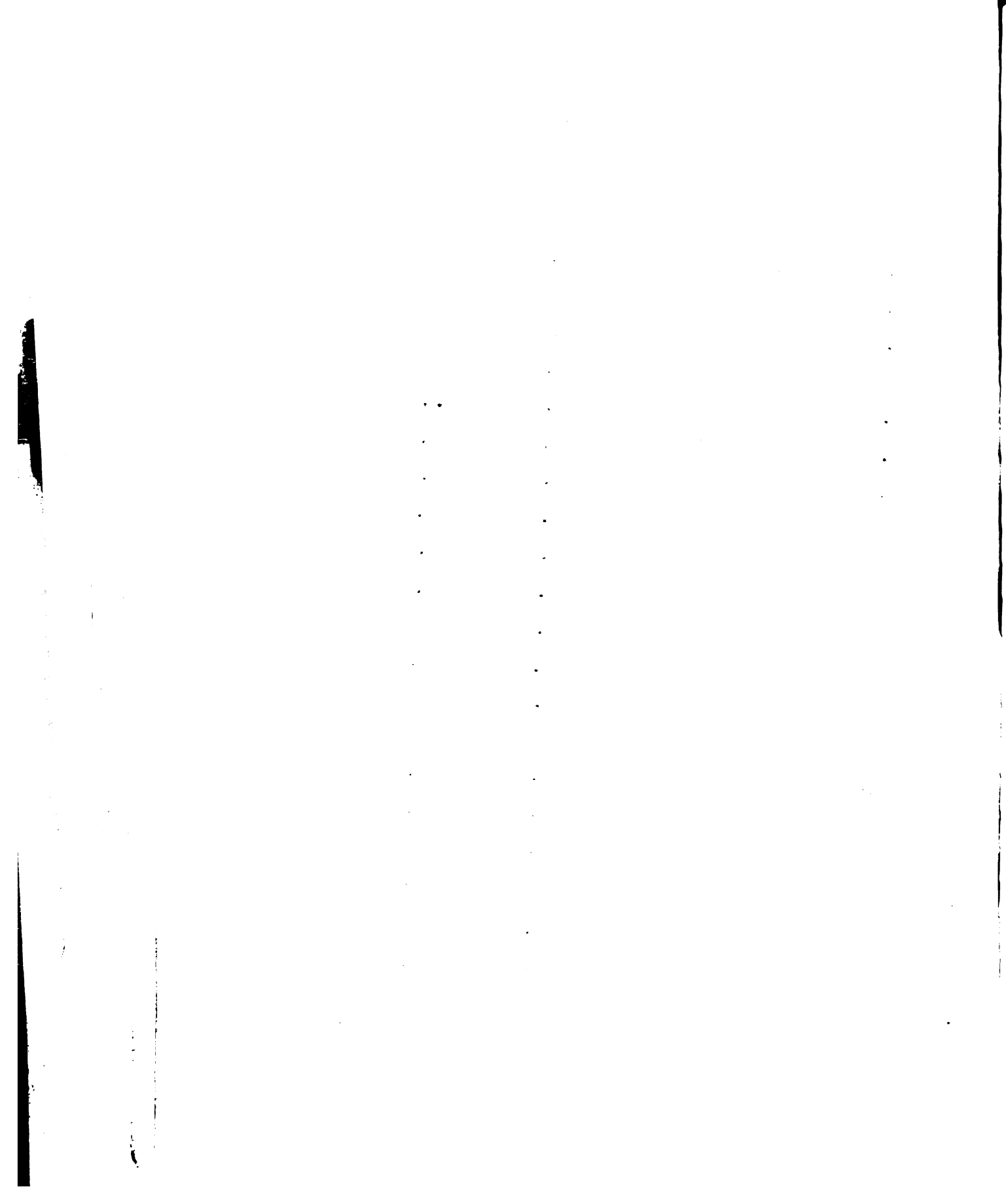
Titration Curves of
Coons & Richards
Nutrient Media.





Changes in uninoculated media after 1-1/2 months.

Goons' medium + Indicator			Richard's medium + Indicator		
Start	Final	Indicator	Start	Final	
2.4	2.4	T.B.	2.	2	
2.4	2.4	" "	2.		
2.8	2.8	T.B.	2.8		
2.8			2.8		
3.2	3.2	BPB	3.7		
3.2	3.2	"	3.7	3.7	
3.8	3.8		4.8	4.8	
3.8			4.8	4.8	
4.2			5.0	5.0	
4.2			5.0	5.0	
5.0	5.0	MR	5.8		
5.0			5.8	5.6	BCP
5.6	Faded	MR	6.4		
5.6	Faded	MR	6.4		
6.4	6.4	BTB	7.0	6.4	BTB
6.4			7.0	7.0	BTB
7.0			7.6		
7.0			7.6	Approx. 6.6	PR more acid than PR reference.
			8.4		
			8.4	6.6	BTB
8.4	8.4	TB			
8.4		T			



The cultures were grown in the dark. About 12 days after inoculation the color and the relative amounts of growth were observed. Tables XII, XIII and XIV summarize these observations.

No observations were made at that time on the black strain of Colletotrichum lindemuthianum. The variant however, responded in an interesting way. Its growth range was between pH 3.2 and 8.4. It grew true to type in the series on Richards medium producing a slimy growth at the beginning, of creamy or white color, from which white aerial mycelial tufts were given off a little later. On Coons' medium, however, it seemed to revert back to the black form. It would have been interesting to study this particular point further and especially to determine whether the black color was due to fruiting or not.

Cladosporium fulvum responded much more readily. The parent form grew well on the media of pH 2.8 - 7.6 producing the characteristic dark olive green color of the submerged growth which is produced on nearly all media at the base of the slant and the growth under the agar and also the much lighter yellowish olive color, characteristic of the aerial growth. A purplish color (Vinaceous lilac, daphne red) was produced towards the alkaline side of the series and this color is characteristically produced on certain media like Oat and Corn Meal Agar and Prune Juice Agar. The same purplish color was observed in the Variant strain also towards the alkaline side. There was a greater variation in the color of the aerial growth on the Richards series, but there was no characteristic distribution, the same color being found on both the acid and the alkaline side. No purple tints were found on either the parent or the variant. On the contrary the buff color that tinges the mycel-



um of the variant/towards the acid side was present throughout the series on this medium. In general, as has been observed by many investigators, fungi tend to shift the reaction of an alkaline substratum towards the acid side and this was the case in the experiments just described. No further observations were made by the writer on this experiment, but after 1-1/2 months final notes were taken by Dr. Coons and these have been incorporated in the tables.

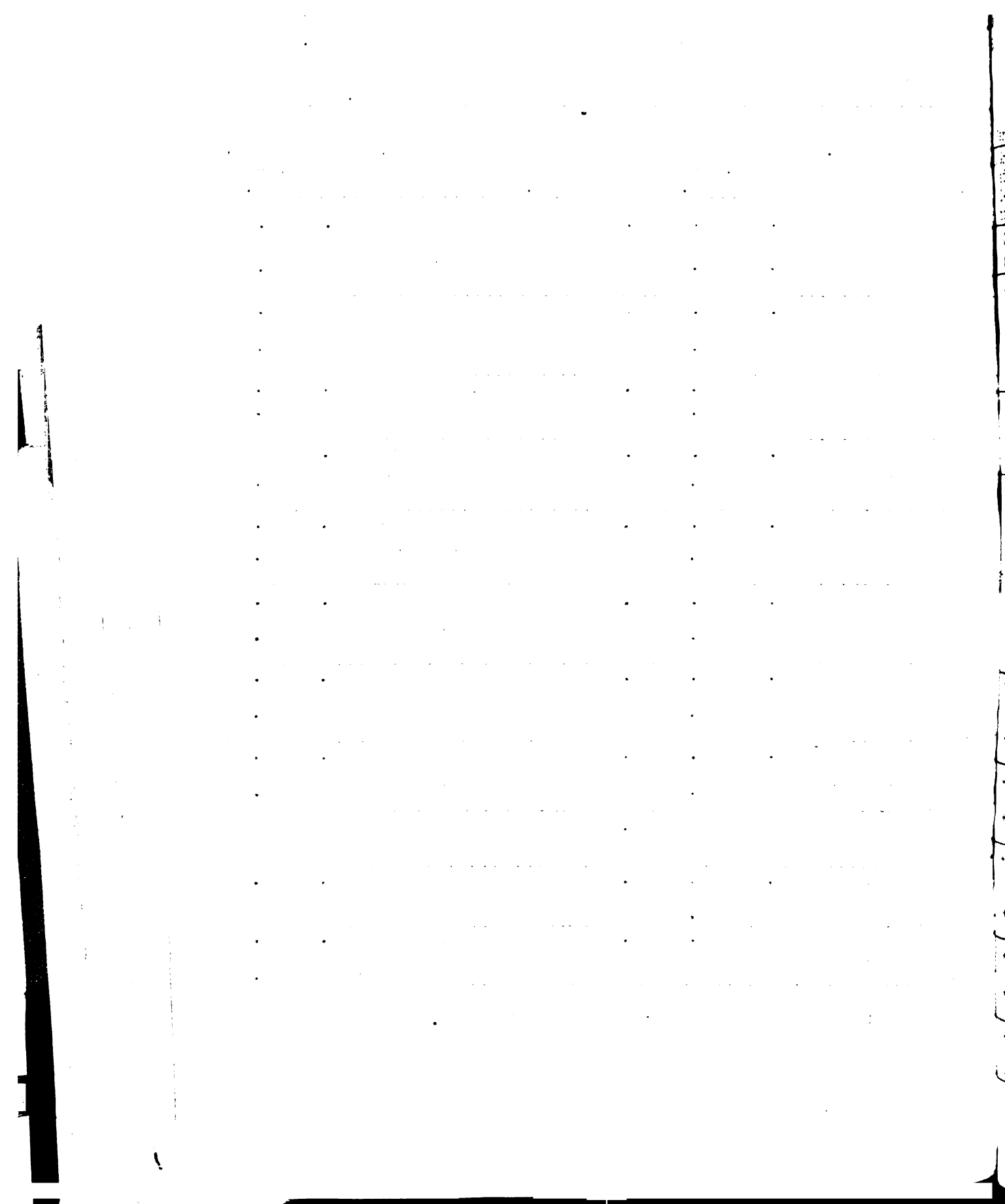
It will be seen that in the case of both species the range of tolerance toward acid and alkaline conditions is practically the same. The effect of the parent and variant strain upon the medium, taking into account the relative growth, is alike. The distinctions in color are maintained; and what is perhaps the most significant, in spite of the great variety of growth conditions presented, there is a complete absence of sporulation in the variant as contrasted with heavy spore production in the parent forms in some cultures.

The differences of the two forms manifested in the previous work seems to be largely maintained.

Comparison of the two strains of *Cladosporium fulvum* in Coons' medium of varying H-ion concentration: Cultures in test tubes.

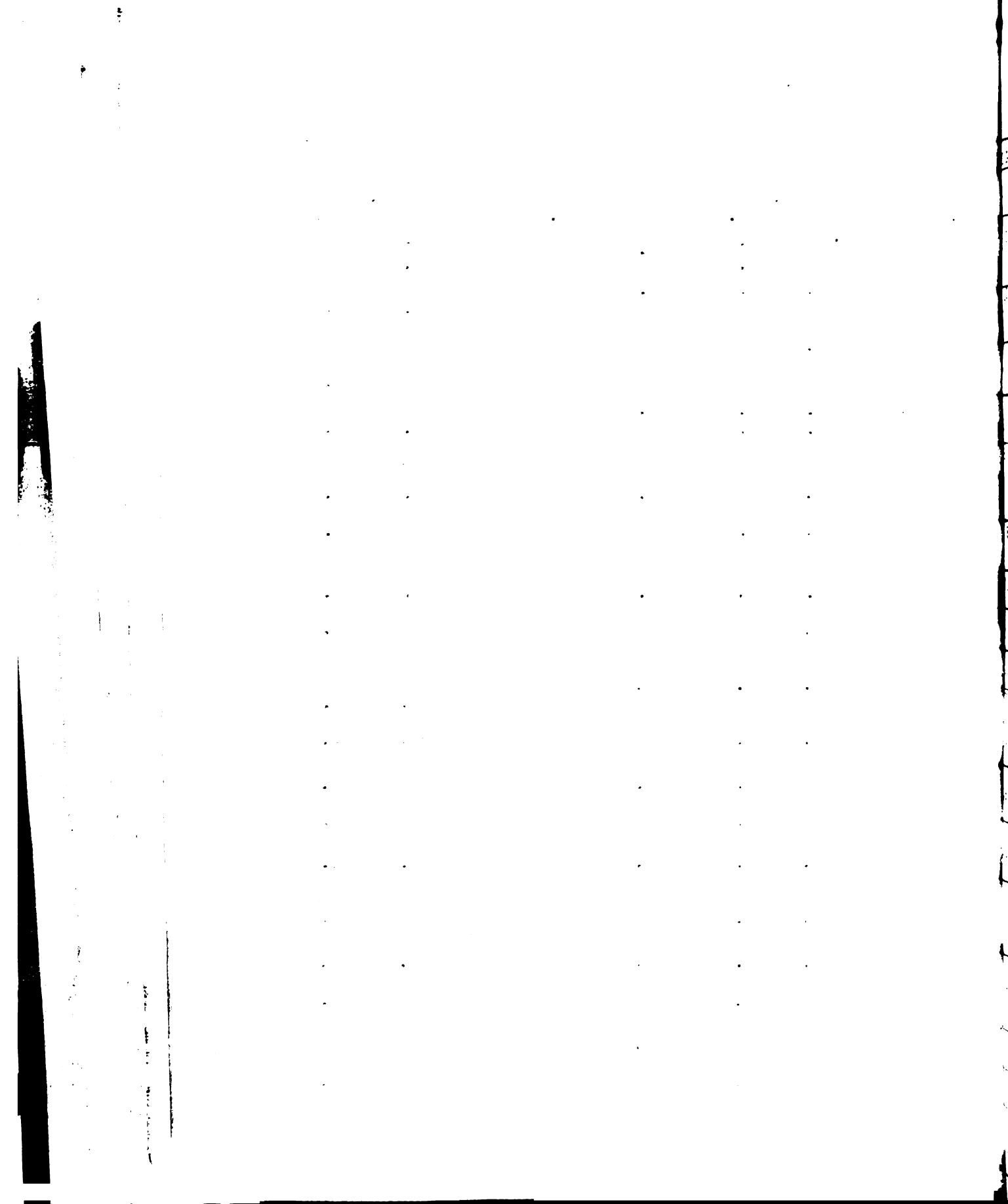
Parent Form					Variant Form.			
Growth after 1-1/2 mo.	Color after 1-1/2 mo.	PH after 12 da.	PH after 1-1/2 mo.	Initial PH	Growth after 1-1/2 mo.	Color after 1-1/2 mo.	PH after 12 da.	PH after 1-1/2 mo.
±	(Merely starting)	2.4	2.4	2.4	±	(Merely starting)	2.4	2.4
±	"	2.4	2.4		±	"		2.4
+	Olive gray	2.8	2.6	2.8	+	Bluish gray		3.0
+	" "		2.8		+	" "		3.4
++	Warm buff		3.2	3.2	++	Light buff	3.2	3.4
++	" "		3.2		++	" "		3.4
+++	Tawny	3.6	4.0	3.8	++	Light buff	3.6	4
+++	"		4.0		++	" "		4.2
++++	Buckthorn [†]	4.2	4.6	4.2	+++	Dark (Submerged)	4.2	4.6
++++	Brown		4.4		+++	"		4.6
++++	Buckthorn ^{##}	5.0	5.2	5.0	++	Light buff	5.0	4.8
++++	Brown		5.2		++	" "		4.8
+++	Buckthorn	5.6	5.6	5.6	+++	Light buff	5.6	5.4
+++	Brown		5.6		+++	" "		5.4
++	Buckthorn [†]	6.4	6.2	6.4	++	Light buff	6.4	6.2
++	" "		6.4		++	" "		6.2
				7.0				
++	Buckthorn	7.2	7.2	7.6	++	Light tan	7.2	7.2
++	Brown		7.2					
+	Russet		7.4	8.4	++	Pinkish	8.4	6.8
	vinaceous				++	white		7.6
						" "		

. white island; ^{##} 4 white islands; [†] 2 white islands.



Comparison of the two strains of Cladosporium fulvum on Richard's medium of varying H-ion concentration: Cultures in test tubes.

Parent Form					Variant Form.				
al	Growth after 1-1/2 mo.	Color	PH after 12 da.	PH after 1-1/2 mo.	Initial PH	Growth after 1-1/2 mo.	Color	PH after 12 da.	PH after 1-1/2 mo.
	0			2.0	2.0	0		2.0	
	0			2.0		0		2.0	
	+	Light Olive Gray	2.8	2.5	2.8	+	(Submerged)	2.8	2.5
	+	"	2.8	2.5		+	Light Gull Gray		2.5
	+	Gray	3.5	3.2	3.7				
	+	Light olive gray	3.5	2.4		+	Pale Olive Gray	3.5	2.8
	++	Tea green	4.6		4.8	++	Ivory yellow	4.6	2.8
	++	"	4.6	2.7		++	Light grayish olive		2.8
	+	Mineral Gray	4.8	2.6	5.0	++	Olive buff	4.8	2.6
	+	"	4.8			++	Vina-seous buff		2.4
	++	Tea green	5.4	2.6	5.8	+++	Ochraceous tawny	5.4	2.8
	++	" "	5.4	2.6		+++	" "		2.4
	++	Olive lake		3.6	6.4	++++	Gray		2.2
	++	" "		2.4					2.4
	++	Light Olive gray	6.6	3.2	7.0	++	Pale olive buff	6.6	2.4
	++	Olive gray	6.6	2.6		++	" "		2.5
	++	Olive gray	7.0	2.6	7.6	+++	Pinkish buff	7.0	2.4
	++	Olive gray	7.0	2.6		+++	" "		2.4
	++	Olive gray		4.1	8.4	+	Pinkish buff		4.4
	++	Olive.		2.5		+	" "		3.5



Comparison of the two strains of *Cladosporium fulvum* on Coons and Richard's media of varying H-ion concentration: Cultures in preparation dishes with filter paper cones.

Parent Form		Richard's Medium			Variant/Form.		
Growth	Color	Spores	No.	PH	Growth	Color	Spores
-			3	2.	-		-
-			4		-		-
Contam.			11	2.8	+	Greenish	-
+	Dark Gray-green	±	12		+	"	-
Contam.			19	3.7	Contam.		
++	Dark Gray-green	+	20		+	Tan	-
+	Blue green	-	27	4.8	+	Tan	-
++	Greenish	+	28		+	"	-
+	Olive green	-	35	5.0	Contam.		
+	"	-	36		"		
+	Olive green	+	43	5.8	+	Pale vinaceous lilac	-
+	Greenish black		44		+	"	-
++++	Olive green	+	51	6.4	++	Buff	-
++++	"	+	52		Contam.		
+++	Olive green	+	59	7.0	++	Buff	-
Contam.			60		++	"	-
+++	Blue green	+	67	7.6	Contam.		
+++	"	+	68		Contam.		
+++	Blue green	+	75	8.4	++++	Dark gray	-
Contam.			76		++++	"	-
Coons' Medium							
-			83	2.4	++++	Greenish	-
+	Grayish	+	84		++++	"	-
+	Dark greenish	+	91	2.8	++++	Grayish black	-
+	Dark Green	+	92		++++	"	-
++++	Light buff	+	99	3.2	++++	Grayish black	-
++++	"		100		++++	"	-
+++	Dark purple	+	107	3.8	++	Light vinaceous lilac	-
+++	"	xx	108		++	Light vinaceous lilac	-
+++	Dark purple	+	115	4.2	++	Light vinaceous lilac	-
+++	"	x	116		+	White,	-
+++	"	xx	123	5.0	+++	Pale vinaceous	-
+++	"	+	124		+++	"	-
+++	"	+	131	5.6	+++	"	-
+++	"	+	132		Contam.		
++++	purple	x	139	6.4	++++	Light vinaceous lilac, buff to	-
+++	purplish-brown	+	140		+++	"	-



continued.

Coona' Medium (Continued)							
Growth	Color	Spores	No.	PH	Growth	Color	Spores.
++++	Purplish brown	x	147	7.0	++++	Deep vinaceous lavender	—
++++	" "	+	148		Contam.		
++++	" "	x	155	7.6	++	Deep vinaceous purple	—
++++	" "	xx	156		++	" " "	—
+	purplish	—	163	8.4	++	" " "	—
+	"	xxx	164		Contam.		

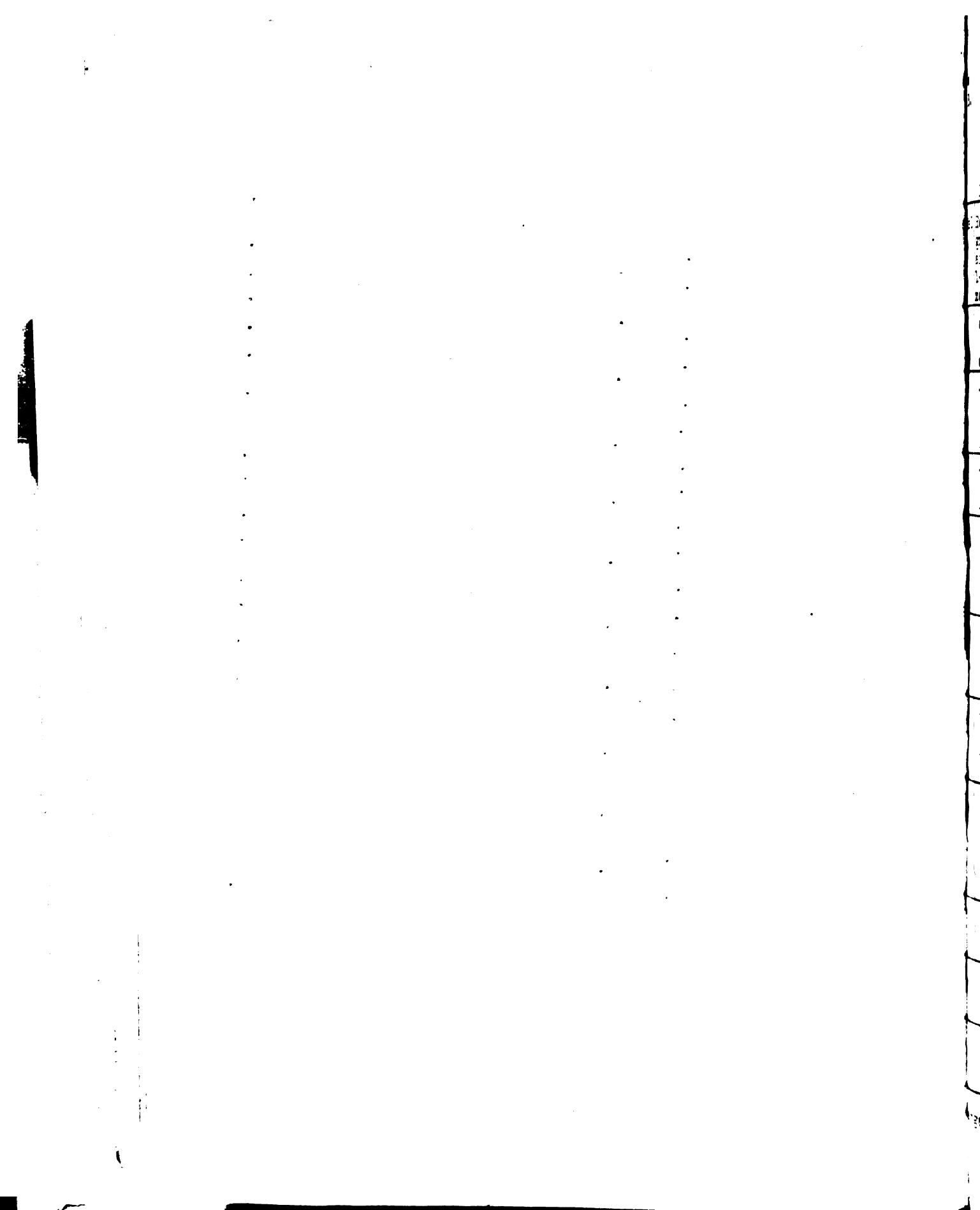
:-White Island.

Comparison of the two strains of Colletotrichum lindemuthianum II when grown on Coons' medium of varying concentrations: Cultures in test tubes.

-61-

Parent Form				Variant Form				
Relative growth after 1-1/2 mo.	Color	Acervuli with spores	pH after 1-1/2 mo.	Initial pH	Relative growth after 1-1/2 mo.	Color	Acervuli with spores	pH after 1-1/2 mo.
0			2.4		0			2.4
0			2.4	2.4	0			2.4
+++	Black	+++	2.8	2.8	+	White [#]	0	3.2
+++	Black	+++	3.6	3.2	+	White [#]	0	3.6
+++	"	+++	3.6		+	White [#]	0	3.5
+++	"	+++	4.4	3.8	-			
+++	"	+++	4.6		++	White [#]	0	4.6
+++	"	+++	4.6	4.2	++	White [#]	0	4.6
+++	"	+++	4.6		++	White [#]	0	4.6
+++	"	+++	5.4	5.0	+	White [#]	0	4.4
0		0	5.0		+++	White [#]	0	5.2
0		0	5.5	5.6	+	White [#]	0	4.8
+++	Black	+++	6.6		+++	White [#]	0	5.4
+++	"	+++	6.2	6.2	++	White [#]	0	6.
+++	"	+++	6.2					
				7.0				
				7.6				
+++	Black	+++	7.6	8.4				
+++		+++	7.2		++	White [#]	0	7.2

arged.

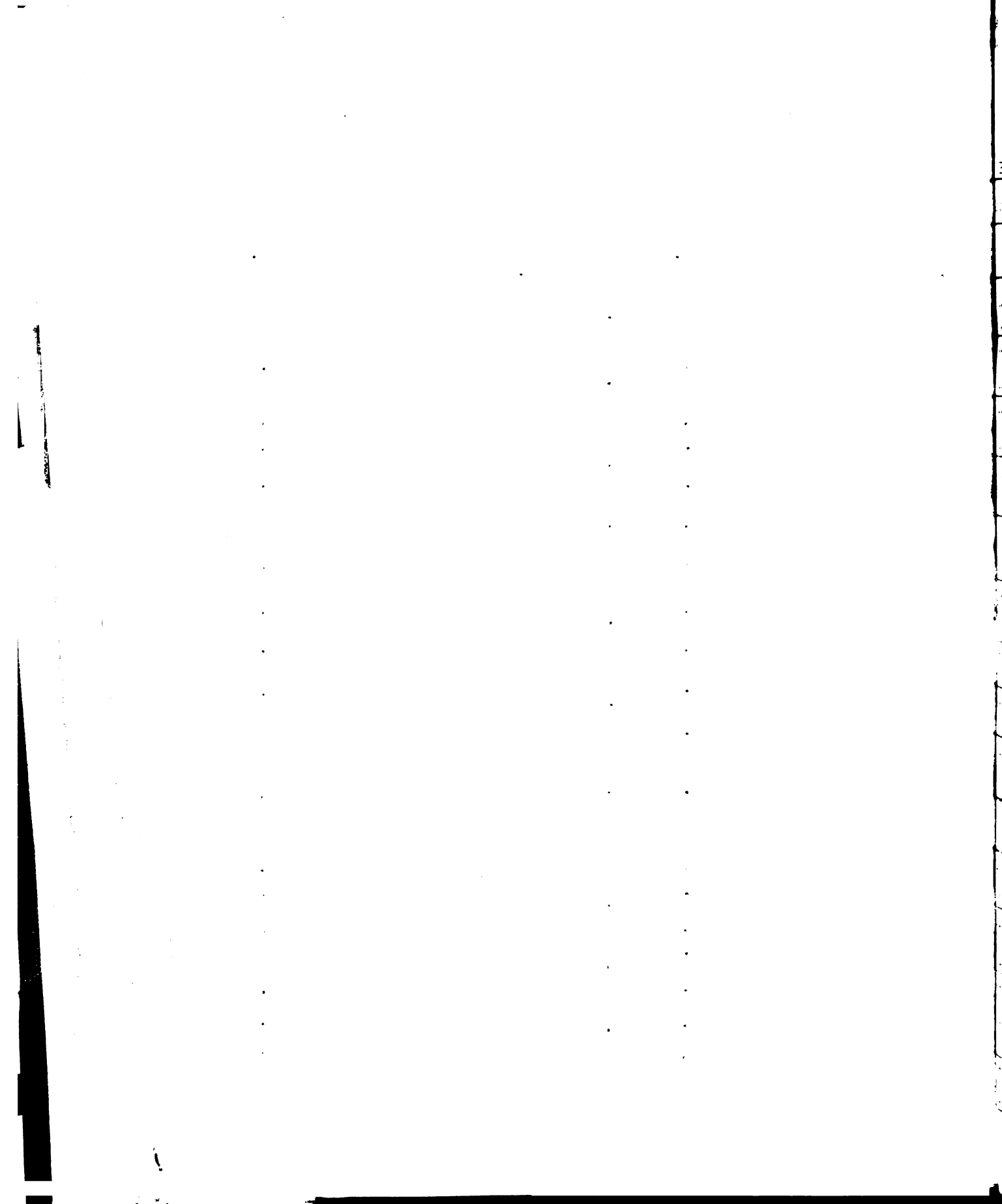


B.

Comparison of the two strains of Colletotrichum lindemuthianum when grown on Richard's medium of varying H-ion contrations: cultures in test tubes.

	Parent Form				Variant/Form				
No.	Relative growth after 1-1/2 mo.	Color	Acervuli with spores	pH after 1-1/2 mo.	Initial pH	Relative growth after 1-1/2 mo.	Color	Acervuli with spores	pH after 1-1/2 mo.
	0				2.	±		0	
	0					0		0	
	+	Not recorded	0	2.6	2.8	+		0	2.8
	0			2.6		+		0	2.8
	+++	Cream	+	4.4	3.7	++	White [#]	0	4.4
	+++	"	+	5.8		++	"	0	5.1
	++	White [#]	0	4.8	4.8	-			
	+++	Shell pink	+	5.7		+++	White [#]	0	5.2
	++	White [#]	+	4.8	5.0	++	White [#]	0	4.6
	+++	Shell pink	+++	6.6		+++	"	0	3.6
	+++	Shell pink	++	6.4	5.8	++	White [#]	0	4.6
	+++	Shell pink	+++	6.4					
	+++	Shell pink but black with acervuli	+++	6.3	6.4	++	White [#]	0	5.4
	+++	" " "	+++	6.3		++	"	0	5.0
	+++	" " "	+++	6.2	7.0	++	"	0	5.8
	+++	" " "	+++	6.4		+++	"	0	4.9
	++	Shell pink	+	5.9	7.6				
	++	" "	0	6.0		++	White [#]	0	5.8
	++	Shell pink	+++	6.6	8.4	+	"	0	6.5
	++	" "	+++	6.2		+	"	0	6.2

merged.



Comparison of two strains of Colletotrichum lindemuthianum II, when grown on Richard's and Coons media of varying H-ion concentration: Cultures in preparation dishes with filter paper cones.

Parent Form		Richard's sol.		Variant Form		
Growth	Color	Spores	pH	Growth	Color	Spores
-			2.	-		
-				-		
+++	Black	+	2.8	-		
+++	"	+		-		
++++	"	+	3.7	Contam.		
++++	"	+		"		
++++	"	+	4.8	Contam.		
++++	"	+		+		
Contam.			5.	Contam.		
++++	Black	+		"		
++++	"	+	5.8	Contam.		
Contam.				+	Creamy	-
++++	Black	-	6.4	++++	Cream	-
++++	"	-		Contam.		
++++	"	-	7.	Contam.		
Contam.				++++	Cream	-
++++	"	+	7.6	+++	Cream	-
++++	"	+		+++	"	-
++++	"	+	8.4	+++	Cream	-
Contam.				+++	"	-
Coons Synthetic Sol.						
+		-	2.4	+	Cream	-
Contam.				+	"	-
++	Greenish black	-	2.8	++++	Dark gray	-
++++	" "	+		++++	" "	-
++++	Black	+	3.2	++++	Grayish black	-
++++	"	+		++++	Jet black in places	-
++++	"	+	3.8	++++	Jet black in places	-
++++	"	+		++++	" "	-

Continued)

Coons Synthetic Sol.(Continued)

t Form				Variant Form		
th	Color	Spores	pH	Growth	Color	Spores.
	Black	+	4.2	+++	Red brown	-
	"	+		++++	Black	-
	"	+	5.	++	Black	-
	"	+		++	"	-
	"	+	5.6	++	"	-
	"	+		++	"	-
	Black	+	6.4	+	Black	-
	"	+		Contam.		
	"	+	7.	+++	Black	-
	"	+		+++	"	-
	"	+	7.6	+++	"	-
	"	+		Contam.		
	"	++	8.4	++	Black	-
	"	++		++	"	-

EFFECT OF TEMPERATURE AND REACTION ON COLOR AND GROWTH.

In connection with another experiment carried on in Berkeley, Calif. both parent and variant strains of Cladosporium fulvum were grown on plated Shive's Best Dextrose agar (79) varying in pH from 4-8 and kept at 30-32° C. & 20° C., and 10° C. The medium was prepared in lots of one liter, brought to the desired H-ion concentration by the addition of acid or alkali, tubed at the rate of exactly 10 cc. per tube and steamed for one hour on three consecutive days. Duplicate plates were poured, allowed to cool and inoculated in the center. They were wrapped in paper and kept at a constant temperature as mentioned above. At intervals the plates were examined for color and rate of growth. The composition of this medium is as follows:

MgSO ₄	2.12 gms.
Ca(NO ₃) ₂	.71 "
KH ₂ PO ₄	1.36 "
FeNO ₃ 5%	1 drop.
Dextrose	20 gms.
Water	1000 cc.

The following table gives the number of cc. of N/5 KOH required to adjust one liter of the medium to a given pH, as determined by Sideris (79).

TABLE XVI.

cc. of N/5 KOH required to adjust 1 Liter of Shive's Best Dextrose Agar to a given pH.

pH	4.0	4.5	5.0	5.2	5.4	5.6	5.8	6.0	6.2
cc. N/5 KOH.	***	.4	.8	1.0	1.8	2.6	5.0	6.0	7.6
pH	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
cc. N/5 KOH	9.2	11.5	14.5	17.5	21.7	25.9	30.8	33.7	36.5

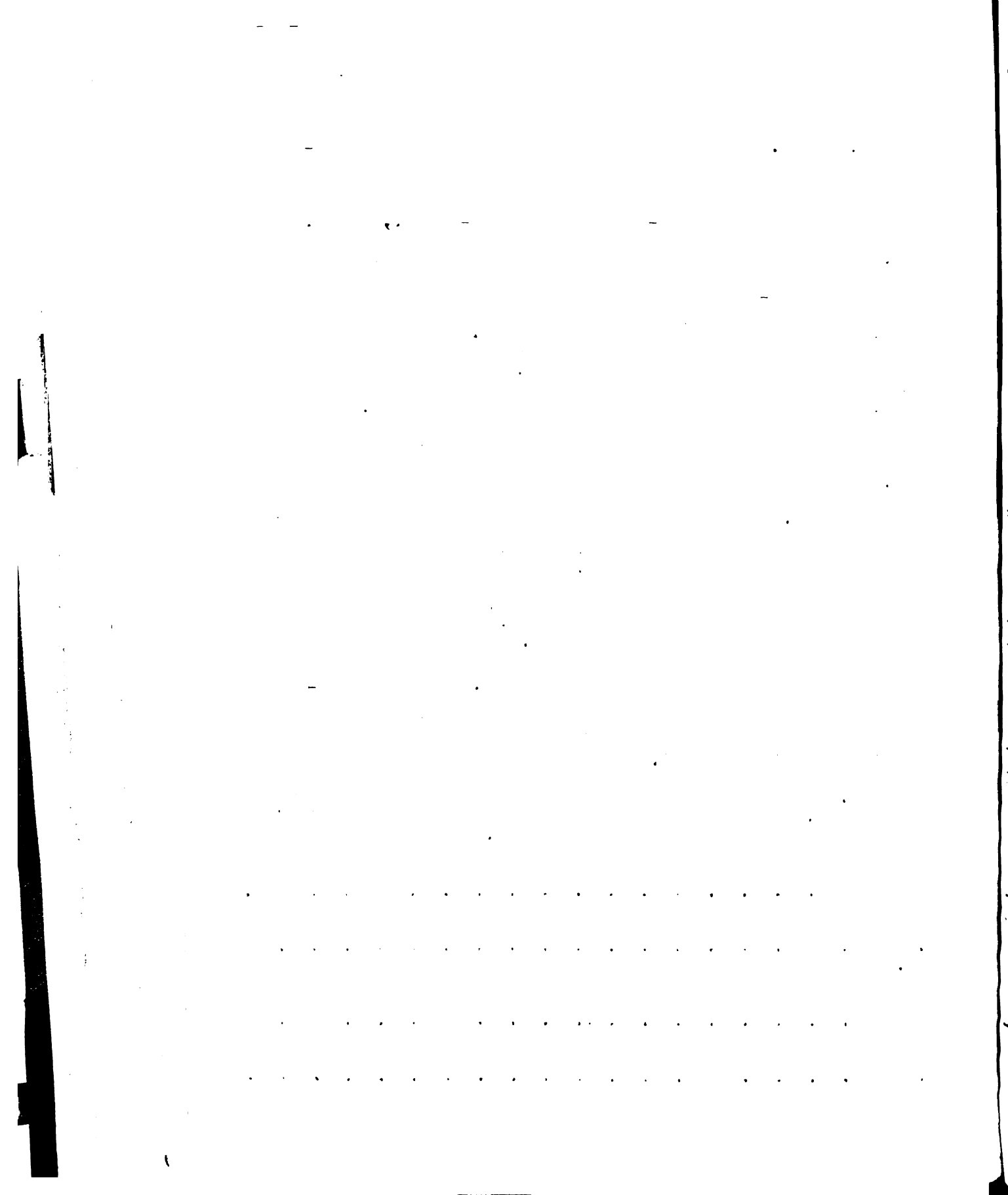


TABLE XVII.

Effect of Temperature and reaction on Color and Growth of the Parent and Variant Strains of *Cladosporium fulvum* grown on modified Shive's Best Medium of varying pH and kept at different temperatures.

Temperature 30-32° C.

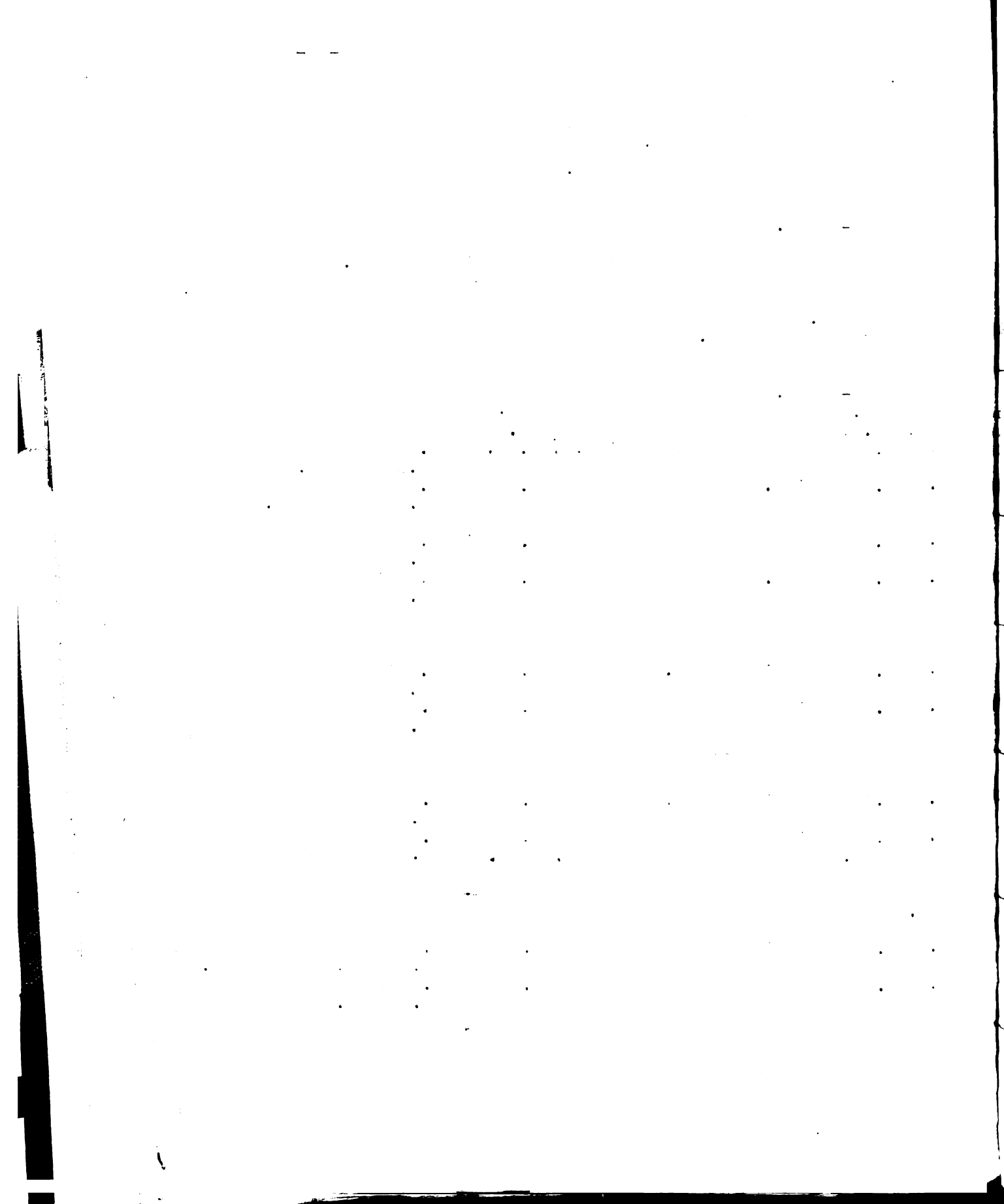
No growth beyond the point of inoculation at which a slight weak growth was observed 54 days after inoculation.

Temperature 10° C.

No growth whatever.

Temperature 20-25° C.

	Diam.	AFTER 3 DAYS	Diam.	AFTER 8 DAYS
pH 4.0	mm.		mm.	
Par.	3.0	Slight growth, whitish.	6.5	Surf. Light olive green Under. Red Brown.
Var.	2.5	White.	5.5	Surf. White Under. Yellow Brown.
pH 5.0				
Par.	1.5	Greenish Brown	4.5	Surf. Light Olive Green Under. Green
Var.	2.0	White.	4.0	Surf. White Under. Dark Yellow Brown
pH 6.0				
Par.	2.0	Greenish Brown.	3.0	Surf. Light Olive green Under. Green
Var.	3.0	White	4.5	Surf. White Under. Dark Yellow brown
pH 7.0				
Par.	2.0	Greenish brown.	6.0	Surf. Light Olive green Under. Purple brown
Var.	2.5	White	4.5	Surf. White Under. Orange Brown
pH 8.0				
Par.	2.5	Greenish Brown	5.5	Surf. Light Olive green Under. Purple, white edge.
Var.	3.0	White	5.0	Surf. White Under. Purple.-



XVII. (Continued)

AFTER 54 DAYS

Surf. Brownish Olive 30
Under. Dull Greenish Black 47
S. White center, clay color edge.
U. Hessian Brown center, Ochraceous Orange edge.

Light Brownish olive 30
S. Dull greenish Black 47
S. Pale Yellow Orange
U. Hazel 14.

Light Brownish Olive 30
U. Dull greenish black 47
S. White center, Ochraceous buff edge.
U. Hazel 14.

S. Light Brownish Olive 30
U. Dull greenish Black 47
S. White-Light Ochraceous Buff.
U. Hessian Brown.

S. Light Brownish Olive 30
U. Olivaceous Greenish Black 47
S. White-Pale ochraceous Buff.
U. Hessian Brown.



Table XVII gives the growth of the fungus (Diameter of colonies in m.m.) and color of the aerial and submerged part, three, eight and fifty four days after inoculation at the three temperatures used.

Makemson (54) found that the temperature limits for best growth of this fungus were rather narrow, the fungus growing best at between 20 and 25° C. The experiment reported above and the fact that the writer experienced great difficulty in keeping this fungus in culture during the summer in Fresno, Calif. (Av. temp. 32° C.), confirm these results. Temperature therefore, did not enter as a factor in this experiment. The variant was found to be a more active grower than the parent strain as indicated by the colony measurements and both parent and variant seemed to grow rather uniformly within the H-ion concentrations limits of the experiment.

Regarding the color, this experiment confirms the results of the previous experiments on the effect of the reaction of the medium. No striking variations were seen even between the extreme ends of the series, in either the variant or the parent except perhaps the development of the purple color in the variant towards the alkaline end as had also been observed in previous experiments.

A repetition of this experiment gave similar results.

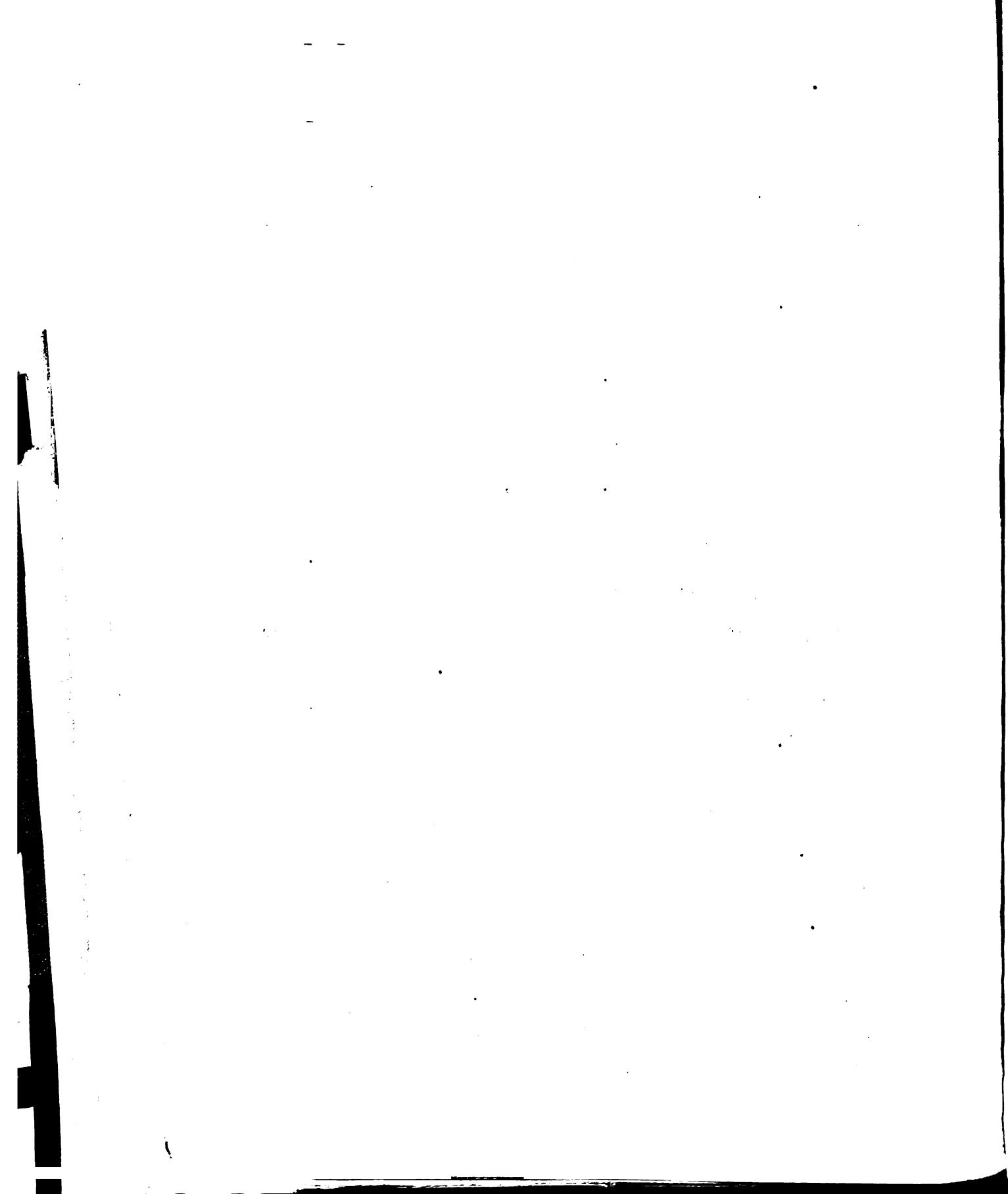


PATHOGENICITY.

The majority of the variations induced through unfavorable environmental conditions have been among the saprophytic fungi and bacteria. Of the spontaneous variations or mutations reported in pathogenic fungi a few do not show reduced pathogenic action while in others the reverse is true and in still others data are lacking.

Edgerton (36) found that the *Glomerella* mutant reported by him grew very slowly on apple. The *Brachysporium* mutant reported by Bonar (12) was not pathogenic on white clover and came from a parent of reduced virulence due to artificial cultivation for many generations. Bennett, who according to Coons (25) investigated white forms developed from a culture of *Phoma apicola* found that they are also attenuated forms. On the other hand Grabill's *Coniothyrium* variant (31) was pathogenic as also were the *Glomerella* variant reported by Dastur (32) and the *Botrytis* reported by Brierley (15). Definite information is lacking in the cases reported by Burger (19), and Stevens (83).

Pathogenicity experiments were carried out using both Parents and Variants of *Cladosporium fulvum* and *Colletotrichum lindemuthianum*. Potted plants of Stone and Beauty Varieties of Tomatoes and Rust Proof Wax Bean were inoculated in the following way. A vigorously growing young culture of each fungus to be tested was macerated in a mortar under aseptic conditions, sterile water added and filtered. Half of the filtrate, a drop of which was examined microscopically for presence of spores or mycelium, was sprayed in each case on



five plants, the other half being used in swabbing the under side of leaves of another set of potted plants. A third set of five plants served as checks. The inoculations were made in the evening, and small flecks of sterile absorbent cotton were placed on the inoculated leaves to prevent rapid evaporation. Each set of plants was kept in a separate moist chamber in the greenhouse until the next morning and were then brought into the greenhouse.

These experiments were all unsuccessful, not a single case of leaf mold or anthracnose being noted on the inoculated plants. This failure of the inoculations was probably due to high temperatures and the heavy white fly infestation of the greenhouse.

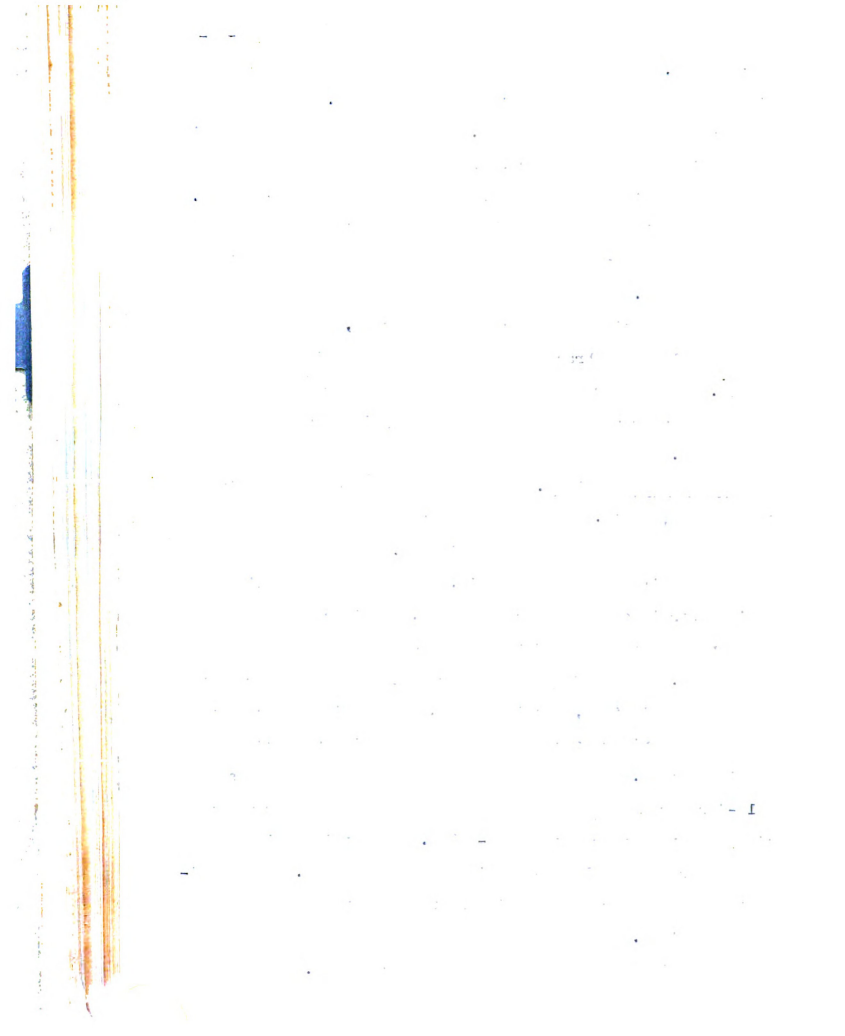
Cladosporium fulvum. The experiments were repeated in 1924 in Berkeley, Calif. Four potted plants of the Stone Variety of Tomatoes were used in these experiments. Two of them were inoculated with the parent strain, and the other two with the variant strain of *Cladosporium fulvum*, the inoculations being made in separate rooms of the laboratory under the usual aseptic conditions. A drop of sterile water placed on the lower or upper surface of a leaflet, was inoculated with a little mycelium from a young vigorous culture and covered with a fleck of sterile cotton. Each plant was put then under a large glass bell-jar for five days and kept exposed to light near a north window of the laboratory at 20-25° C. After this period the plants were gradually brought into the Greenhouse. The experiment was exactly repeated using four potted plants of the Beauty Tomato Variety.

These inoculations were entirely successful. Both the

parent and the variant produced spots at every point of inoculation, more than thirty spots being counted in each case. No spots were seen at other than the inoculation points and although these plants together with many control plants were kept in the same greenhouse there was no natural infection. The spots produced by the parent strain were all characteristic of the disease as described by Makenson (54). There was an olivaceous growth on the under side of the leaf with an abundance of spores characteristic of the fungus. The upper surface of the leaf in the infected area turned yellow and became dry. The spot spread irregularly from the point of inoculation.

The spots produced by the variant strain were elliptical well defined in appearance, rather dry with yellowish flakes on the surface. There was no mycelial growth on the under side of the leaf. Free hand sections through the infected portion of the leaf showed the presence of mycelium throughout the leaf tissue. Five plates were poured from leaves infected by the variant using Czapeck Dextrose Agar for Medium. In every case the white form of *Cladosporium* grew on these plates. Similarly infected leaves were placed in moist chambers. The white variant grew from the margin of every spot. Plates from tissue infected by the parent strain showed an abundance of growth of the typical *Cladosporium fulvum*.

Plate 12 shows leaflets of Tomato artificially infected with both forms of *Cladosporium*. Isolations were made from the part of the leaf infected both by the parent and the variant strains and reinoculations were made on potted Beauty Tomato plants in



parent and the variant produced spots at every point of inoculation, more than thirty spots being counted in each case. No spots were seen at other than the inoculation points and although these plants together with many control plants were kept in the same greenhouse there was no natural infection. The spots produced by the parent strain were all characteristic of the disease as described by Makemson (54). There was an olivaceous growth on the under side of the leaf with an abundance of spores characteristic of the fungus. The upper surface of the leaf in the infected area turned yellow and became dry. The spot spread irregularly from the point of inoculation.

The spots produced by the variant strain were elliptical well defined in appearance, rather dry with yellowish flakes on the surface. There was no mycelial growth on the under side of the leaf. Free hand sections through the infected portion of the leaf showed the presence of mycelium throughout the leaf tissue. Five plates were poured from leaves infected by the variant using Czapeck Dextrose Agar for Medium. In every case the white form of *Cladosporium* grew on these plates. Similarly infected leaves were placed in moist chambers. The white variant grew from the margin of every spot. Plates from tissue infected by the parent strain showed an abundance of growth of the typical *Cladosporium fulvum*.

Plate 12 shows leaflets of Tomato artificially infected with both forms of *Cladosporium*. Isolations were made from the part of the leaf infected both by the parent and the variant strains and reinoculations were made on potted Beauty Tomato plants in



bloom. The methods used were those described above, except that inoculations were made on both the upper and lower sides of the leaflet. No spots were produced from upper surface inoculations. Typical spots similar to those described above developed on all (fifteen in number) of the lower surface inoculations. Inoculations on the calyx and stem of flowers produced characteristic spots. Small tufts of white mycelium were found at the margin of the spots produced by the variant. Such tufts were examined under the microscope for spores. There were no typical spores except a few spore like bodies believed to be ohlamydospores. The mycelium was thin, densely branched, and purple in color.

Dastur (32) found that passing the variant form of *Glomerella* through its host restored its ~~lost~~ spore-producing power, but this was not evidently the case with *Cladosporium fulvum*, since it did not produce spores even after the second passing through its host. From the experiments described above it becomes evident that both the parent and variant are parasitic on the tomato plant, but growth on the host did not ^{restore} the lost properties of color and fruiting of the variant. Bonar (12) working with *Brachysporium* which had lost its pigment but not the power of fruiting found that the variant was not parasitic and the parent had lost the greater part of its power of parasitism through prolonged cultivation on artificial media. This is not the case with the parent form of *Cladosporium fulvum*, which infected every leaf inoculated although it had been under artificial conditions for over four years.

Colletotrichum lindemuthianum. Golden Wax Bean plants were inoculated with the parent and variant form of Colletotrichum lindemuthianum Exp. Sta. Strain II, using spores or bits of mycelium in the same manner as in the tomato plant inoculations. Inoculations were made on both the upper and the lower surface of the leaves. No spores were formed on any of the upper surface inoculations or on the control leaves. The results were not so definite as in the case of *Cladosporium*. The leaflets inoculated with the white form of *Colletotrichum* turned yellow but no definite spots developed except in one case where typical anthracnose spots were observed. No spores were found. The leaflets inoculated with the black form of *Colletotrichum* showed typical spots of anthracnose with an abundance of spores.

DISCUSSION AND CONCLUSION.

As mentioned at the beginning of this paper, the proposed experimentation, that was intended to procure the arguments for a discussion of the whole problem, was not entirely carried out. Such experiments as are recorded here, preliminary in their nature as they are, do not throw light on this complicated and much discussed problem. Perhaps it would be of interest to give here an account of further experiments that the writer had in mind for the same reasons that the present experiments are recorded - to form the basis of further work.

These experiments are outlined chiefly with the fungus *Cladosporium fulvum* in mind. They could be varied to apply to the other fungi under investigation. And first, a fundamental study of the nutrition of the present fungus is necessary to

furnish the basis for nutritional work. Nutrition is credited by De Vries as the cause of all modal or environmental modifications which are deviations of varying magnitude from the mean and have been found to yield to selection in changing the mean of a specific character. Brierley in 1918 (14) finds also that modal variations are directly and constantly induced by particular substrata. The present investigations show that the strain of Cladosporium fulvum used in these experiments is in the state of mutability according to De Vries, since the white variant has been given out frequently irrespective of conditions of growth. For the nutritional experiments a strain should be found not in a mutating state and this should be compared with the present strain and an attempt made to induce mutability in it. Further experiments along this line would be called for by the progress of the work. Besides nutrition other environmental factors should be investigated in order to corroborate evidence presented by other investigators working with other fungi. The effect of poisons, acids, alkaloids, metabolic products etc., the effect of hydrogen ion concentration kept constant by frequent adjustments throughout the course of the experiment, the effect of light, temperature, controlled humidity should be carefully studied. These should be studied in order ~~in~~ 1. to induce mutability in an otherwise non-mutating strain, 2. to find out if any of these factors regulate the appearance of variants in an ordinarily mutating strain, 3. to determine their effect in changing the mean of any specific character. In the case under investigation a study into the pigmentation of Cladosporium fulvum is necessary.

From the experiments in hand one conclusion could be drawn safely. The environmental modifications tried, exercise an



effect on the color and fruiting of Cladosporium fulvum, they cannot, however, induce the type of variations ordinarily given out at unprognostigated and irregular intervals by the strain under investigation.

One cannot but agree perfectly with De Vries (87) that "nothing is more variable than the meaning of the word variability." (l.c. p. 43) and perhaps the word mutation be substituted for variability. At the beginning of this paper a series of interpretations of the meaning of the term by various authors and by the formulator of the whole theory was given. This list can be extended, but without any particular benefit. The confusion has led several writers to avoid altogether the use of the term. Morishima (58) in 1921 reporting several cases of adaptive changes in bacteria, ^{see 1445} B.typhosus in particular, thinks that the term mutation should be used only with higher plants and should not be introduced into bacteriology "for the bacteriologist, who studies his species not only from the morphological point of view, but also with regard to biochemical and immunological reactions, and who observes not a few generations only, but hundreds and thousands of generations, would almost surely have to modify the conception of the term in such a manner as to cause confusion to the botanist. It, therefore, seems advisable to leave the term mutation to the botanist and, for the present at least, to speak of atypical varieties of bacteria or simply variants." The present writer has also avoided the term mutation for similar reasons. Stevens (83) uses the term saltation with a new meaning to cover variations in non-sexual generations of fungi. Chaudhuri (21) follows his example for the same reason.

Brierley (15) strongly objects to the use of the term mutation "It is not the possibility of mutation in the fungi (and bacteria) that is here denied but rather the compulsion to accept that interpretation of the evidence as it now stands". He rejects the use of the term on the grounds that there is no assurance of genetic purity in asexual fungi. Jennings (45) in 1911, Blakeslee (8) in 1920, Shear and Wood (76) in 1913, Brierley (15) in 1920, and Stevens (83) in 1922 agree that in single spore cultures of asexual fungi we deal with pure line individuals. The term mutation has been applied by De Vries to germinal ^{variations}, arising ^{independently} of environment, remaining constant afterwards, in a rarely self-fertilized plant grown from seed, the genetic purity of which has been seriously attacked by Bateson and other geneticists and proven to be a hybrid whose segregation is checked by balanced lethals as it has been recently explained by Bradley Davis (33). The modern use of the term, however, presupposes according to Brierley (15) and Davis (33) an absolute certainty of genetic purity. In the homozygous asexual generations of fungi coming from single spores and carried in culture for countless generations there is scarcely any chance of genetic contamination. Anastomosing in single spore cultures cannot be accounted as destroying the genetic purity. Shear and Wood (76) discussing the question of ~~genetic~~ heredity and variation in *Glomerella* say "there is no reason to believe that the mendelian theory is involved or heterozygosis takes place as there is no union between nuclei of different individuals and species. If there is any union between cells or nuclei of a single spore organism there is nothing new coming out of the union, no new characters transmitted,,,,,it is hard to see how two closely related

1. The first part of the document is a letter from the President of the United States to the Congress, dated January 1, 1861. It is a very important document, as it sets out the policy of the new administration.

2. The second part of the document is a report from the Secretary of the Treasury, dated January 1, 1861. It contains a detailed account of the financial state of the country at the beginning of the year.

3. The third part of the document is a report from the Secretary of the Interior, dated January 1, 1861. It contains a detailed account of the state of the public lands and the progress of the various departments under his control.

4. The fourth part of the document is a report from the Secretary of the Navy, dated January 1, 1861. It contains a detailed account of the state of the navy and the progress of the various departments under his control.

5. The fifth part of the document is a report from the Secretary of the War, dated January 1, 1861. It contains a detailed account of the state of the army and the progress of the various departments under his control.

6. The sixth part of the document is a report from the Secretary of the State, dated January 1, 1861. It contains a detailed account of the state of the foreign relations of the United States and the progress of the various departments under his control.

7. The seventh part of the document is a report from the Secretary of the Education, dated January 1, 1861. It contains a detailed account of the state of the public schools and the progress of the various departments under his control.

nuclei in the same individual or cell could add essential characters to the organism. Individuals from single spores must be considered homozygous..." "Whatever differences the progeny of such individuals might show would evidently be due to Mutation or some other internal cause, such as the renewed expression of latent hereditary characters."

Brierley (15) rejects the possibility of a mutative origin of variants in the fungus studied by him on the grounds of contaminations by anastomosis in some remote time in the life of the strain before its artificial cultivation. Although conceivable, it is hard to believe of an interspecific or other anastomosis in *Cladosporium* or other fungi that would so modify the nuclei of a cell and build up a system of balanced lethals covering such a modification for many generations, finally breaking up repeatedly into the variation observed. It would particularly be hard to think of such a happening in the variation recorded in this paper, viz. the loss of a single character, that of fruiting, which apparently is accompanied by the loss of the olive-brown pigment character. It is more probable to think of such a case as a retrogressive mutation (mutation by loss) or class it with the vegetative variations known as sports or bud variations which De Vries also classes with Mutations. The doubt of a genetic contamination by anastomosis fades before the doubts of genetic purity in any pure line of higher plants where contamination by cross fertilization and hybridization is much more probable than contamination by anastomosis, in non-sexual plants.

Of the variations in fungi reported in literature the one



nearly
most Δ approaching the one described in this paper is the
white non-fruiting variation reported by Crabill (31) in 1915
in Coniothyrium pyrinum Sheld. This fungus is pathogenic on apple
the white strain being also pathogenic. The loss of the olive-
brown color is accompanied by a loss of fruiting. It has been
obtained several times and has been constant. Environmental
modifications could not induce its throwing off. All these
correspond with the variation of Cladosporium fulvum and
Colletotrichum lindemuthianum reported in this paper.

S U M M A R Y .

Albino forms with a loss of fruiting are reported in
Cladosporium fulvum and Colletotrichum lindemuthianum.

A comparison of the growth of these forms along with
their parents on different standard media, and microscopi-
cally is made.

The parents and variants are grown on a series of media
arranged according to the triangular system for the purpose
of studying the effect of nutrients on color production and
variation. Color was found to vary in Cladosporium fulvum
under the conditions of the experiment. The other fungi used
did not respond.

Light was found to have no effect on variability and color
of Cladosporium fulvum.

Both parents and variants of C. fulvum and C. lindemuthia-
num were grown on Coons' and Richards liquid media ranging in
pH from 2-8.4 in order to study the effect of pH on variability
and color. Both strains of the two fungi were found having a



wide range of growth and varied somewhat in color under the conditions.

The temperature limits of both the parent and the variant of Clad. fulvum are found between 20 and 25° C. No striking variations in color were observed on growing this fungus on solid media varying in pH from 4-8.

Both the parent and the variant of Clad. fulvum and Coll. lindemuthianum were found to be virulent parasites on their respective hosts; passing through the host did not restore the lost characters of the variants.

A review of literature and a discussion of the possibility of mutations in asexual fungi is given.

* * * * F I N I S * * * *

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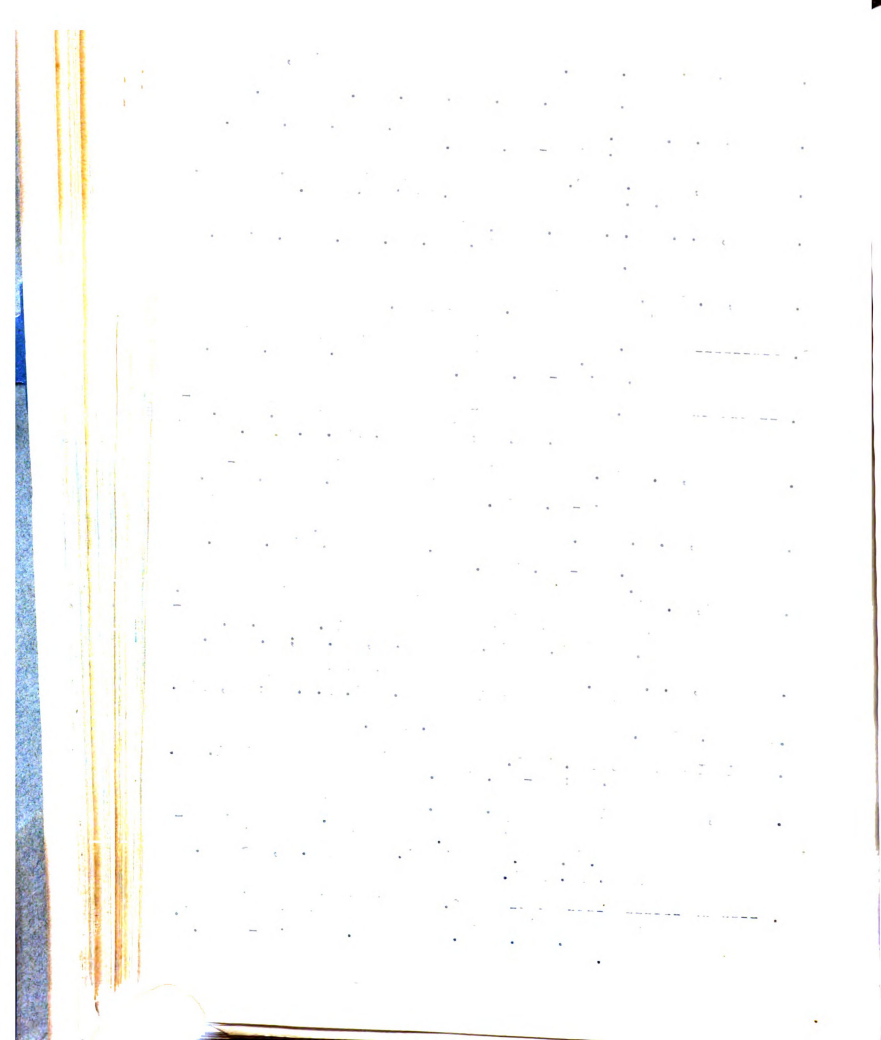
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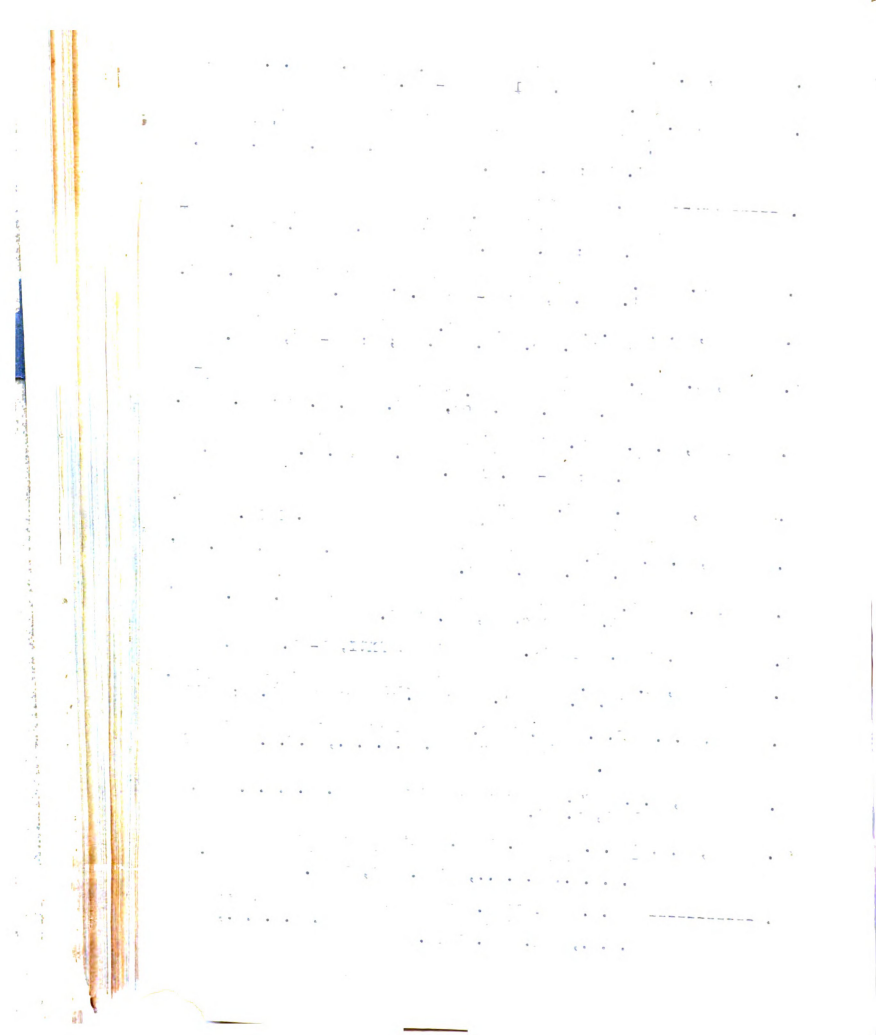
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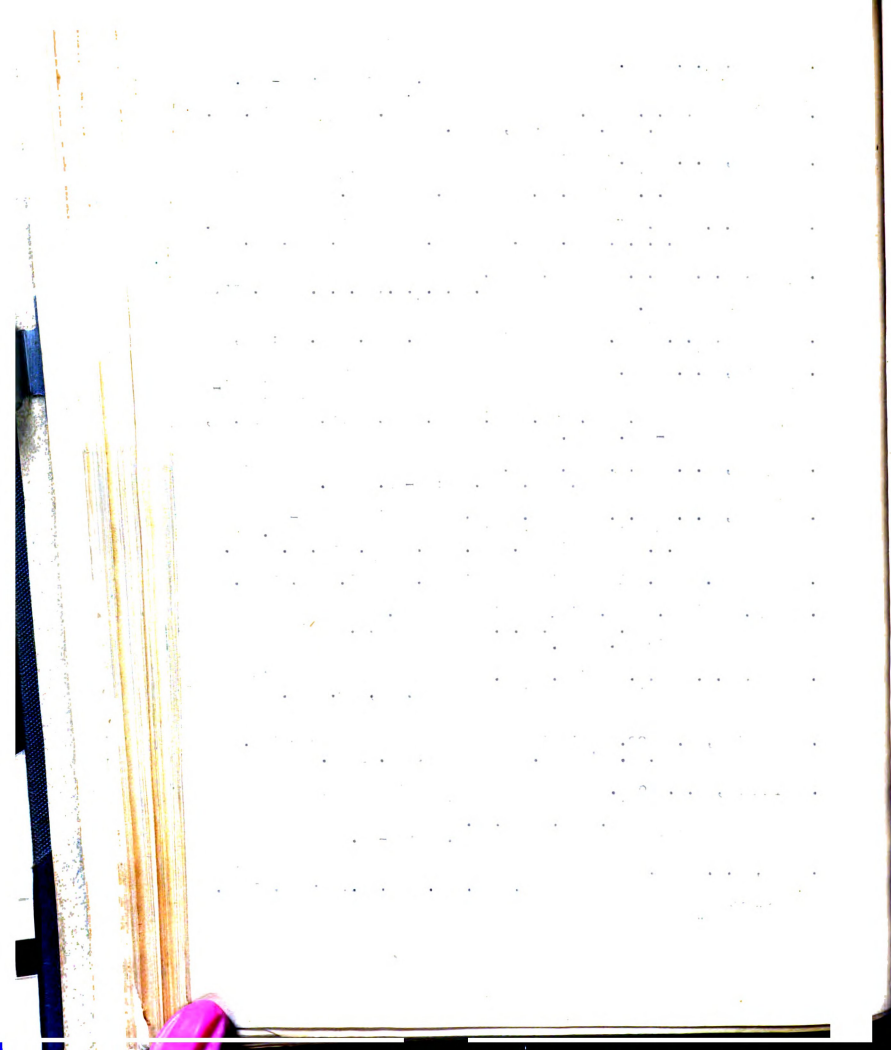
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Achromatic Variations in Pathogenic Fungi.

LEGENDS FOR PLATES I - XII.

- Plate I.**
- Fig. 1. a.** Septoria apii on Corn Meal Flask:
appearance of white tufts of
mycelium.
- b.** Cladosporium fulvum on Corn Meal
Flask. First appearance of the
variant form.
- Fig. 2. a.** Colletotrichum lindemuthianum Exp.
Sta. Str. II. Culture on Corn
Meal Flask showing the appearance of
variations.
- b.** Colletotrichum lindemuthianum Exp.
Sta. Str. II. Normal culture on
Corn Meal.

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PLATE I.





Plate II. A. Cladosporium fulvum. Parent Strain.

A. Mycelium.

B. Conidiophores and Conidia.

B. Cladosporium fulvum. Variant Form.

C. Mycelium.

D. Chlamydospores.

Camera lucida drawings. x 1100

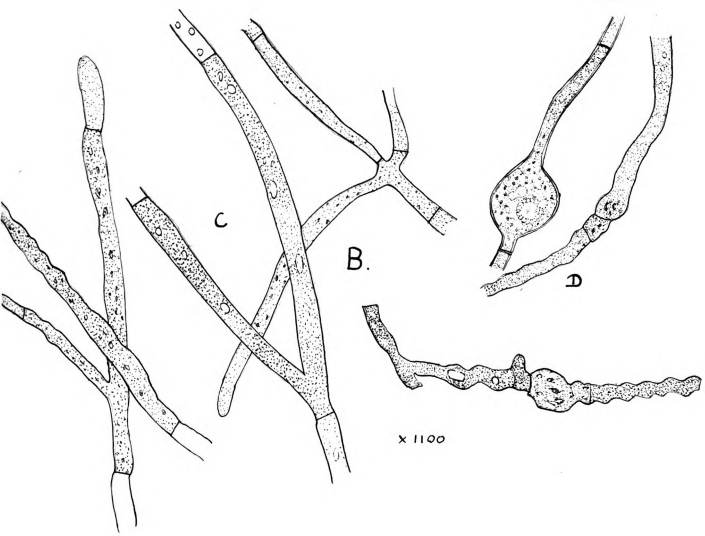
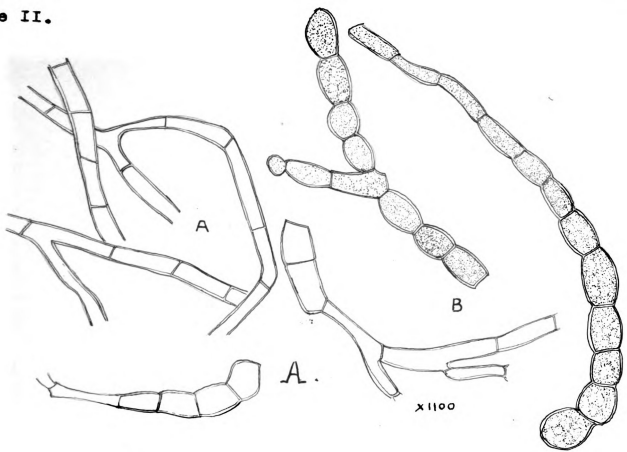
1. General Information

A. Location
B. Geographical Position

2. Geographical Position

A. Location
B. Geographical Position

General Information



- Plate III. a. Colletotrichum lindemuthianum. Culture of
the variant form on Corn Meal.
- b. Cladosporium fulvum. Culture of the parent
form on Steamed Rice.
- c. Cladosporium fulvum. Culture of the variant
form on Corn Meal.

a. Col. William H. Hall
b. Col. William H. Hall
c. Col. William H. Hall
d. Col. William H. Hall
e. Col. William H. Hall

Plate III.



Plate IV. Septoria apii. Growth of the fungus on Coons'
Medium varied according to the triangular
system. Complete set of cultures arranged
as per diagram on page 34.

1
The following is a list of the names of the persons who
were present at the meeting of the Board of Directors
of the American Red Cross, held on the 1st day of
January, 1917.

Plat^o IV..



Plate V.

Colletotrichum lindemuthianum. Exp. Sta. Str. I.
Growth of the fungus on Coons Medium varied
according to the triangular system. Complete set
of cultures arranged as per diagramm on page 34.

1. The first part of the report is devoted to a description of the work done during the year. It is divided into two main sections: a general summary of the work and a detailed account of the results of the experiments.

Plate V.





Plate VI.

Colletotrichum lindemuthianum. Exp. Sta. Str. II.
Growth of the fungus on Coons Medium varied in
composition according to the triangular system.
Complete set of cultures arranged as per diagram
on page 34.

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Plate VI.



Plate VII.

Colletotrichum lindemuthianum Exp. Sta. II.
Growth of the fungus on Coons Solution varied
in composition according to the triangular system.
Complete set of cultures as per diagram on page 34,
and descriptions in Table VI.

10-11-71

In the event of a fire, the fire department
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If a fire occurs, the fire department should be
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If a fire occurs, the fire department should be
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Plate VI.

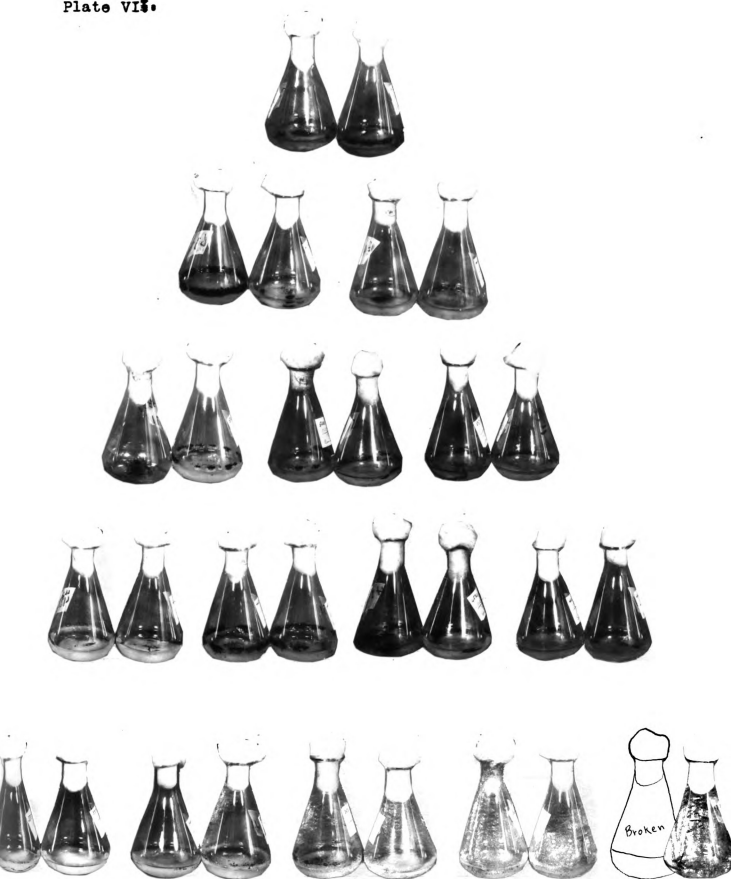


Plate VIII.

Cladosporium fulvum. Parent form grown in light
on Coons medium varied in composition according to
the triangular system.

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Plate VIII.



Plate IX.

Cladosporium fulvum. Parent from grown in the
dark on Coons Medium varied in composition
according to the triangular system.

Plate IX.



Plate X.

Cladosporium fulvum. Variant form grown in the
dark on Coons Medium varied in composition
according to the triangular system.

Plate X.



Plate XI.

**Cladosporium fulvum. Variant form grown in light
on Coons Medium varied in composition according
to the triangular system.**

Plate XI.



Plate XII.

Fig. 2. a and b. Cladosporium fulvum parent form.
Under side of the leaflets showing
typical infection produced by
artificial inoculation.

c. Upper side of the leaflet showing
type of spot.

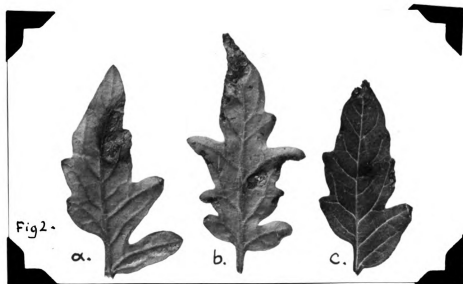
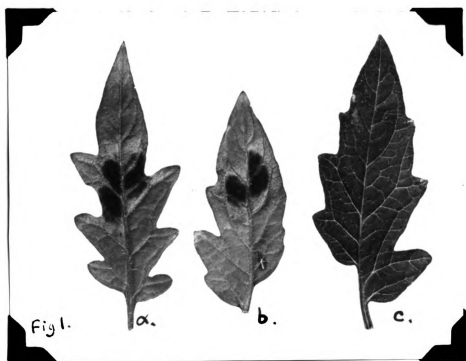
Fig. 2. a and b. Cladosporium fulvum variant form.
Under side of leaflets showing
type of infection.

c. Upper side of leaflet showing
type of spot produced.

10-11-12

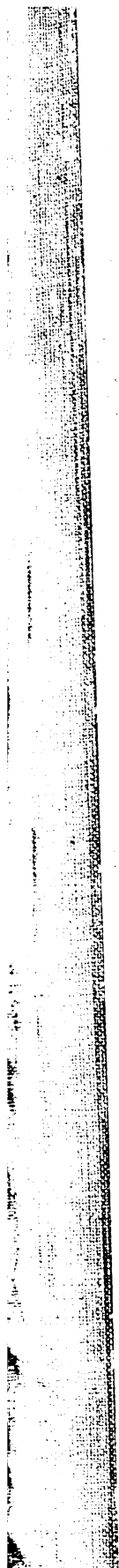
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5. The fifth part of the report
describes the state of the
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economy.

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