

STUDIES ON A FUNGUS OF THE GENUS.

STILBELLA

by

Vathana Phokthavi

A thesis submitted to the Faculty of Graduate Studies and
Research in partial fulfilment of the requirements
for the degree of Master of Science

Plant Pathology,
Macdonald College of
McGill University,
Quebec.

August, 1974.

ABSTRACT

M.Sc.

Vathana Phokthavi

Plant Pathology

STUDIES ON A FUNGUS OF THE GENUS STILBELLA

An stilboid fungus is described which is now assumed to be a new species of the genus Stilbella Lindau, Family Stilbellaceae, Class Deuteromycetes. It was isolated from white clover, Trifolium repens L., which were floated or partially submerged in water.

Its hyphae are narrow, septate and hyaline. Conidio-phores are simple or branched and undistiguated. Two kinds of conidia are produced: macroconidia having the sizes 1.5 - 6.4 (width) x 11.5-18.0 (length) micrometers; and microconidia 1.5-5.7 (width) x 3.5-9.5 (length) micrometers. Hyaline hyphae aggregate into erect columns of synnemata, up to 2 mm tall, with slimy heads of yellowish conidia.

The optimum temperature for growth and sporulation was 25°, on czapek solution agar, at pH 5.6 under the experimental conditions. Zonation of the colony was induced by alternating light-dark periods. The fungus was weak when grown in competition with the other fungi tested. Morphological abnormalities were induced by volatile metabolites produced by the bacteria tested. This stilboid fungus had a wide range of host plants. It was present in the cortical tissues of plant roots without causing any visible evidence of disease.

RESUME

TRAVAUX SUR UN CHAMPIGNON DU GENRE STILBELLA

Un champignon stilboïde est décrit comme pouvant être une nouvelle espèce du genre Stilbella Lindau, de la famille des Stilbellaceae, de la classe des Deuteromycètes. Le champignon a été isolé du trèfle blanc (Trifolium repens L.) mis à flotter ou en partie submergé dans l'eau.

Ses hyphaes mycéliens sont minces, cloisonnés et hyalins. Les conidiophores sont simples ou ramifiés ou indistincts. Deux sortes de conidies sont formées: les macroconidies mesurant 1.5-6.4 micromètres de large sur 11.5-18.0 de long, et les microconidies mesurant 1.5-3.7 de large sur 3.5-9.5 de long.

Les hyphas hyalins s'unissent pour former des synnemas sporifères en forme de colonne pouvant atteindre 2 mm de haut, et possédant une tête visqueuse de conidies jaunâtres.

La température optimale pour la croissance végétative et la sporulation est 25° sur le milieu czapek gélosé, au pH 5.6. L'alternance de périodes d'éclairement et de périodes sombres produit des colonies zonées. La croissance du champignon est faible en présence de celle d'autres champignons. Sa morphologie devient anormale en présence des substances volatiles émanant des cultures de bactéries que nous avons étudiées. Ce champignon stilboïde se retrouve sur plusieurs plantes-hôtes. Il envahit le cortex des racines sans pour cela causer des signes évidents de maladie.

ACKNOWLEDGEMENTS

The author is deeply indebted to Professor R.H. Estey, chairman, Dept of Plant Pathology, Macdonald College, for his supervision throughout the course of these experiments and the writing of this thesis, to Professor R.L. Pelletier for translating the abstract, to Dr. D. Swales and Professor D.W. Woodland for identifying the collected plant specimens, and to Professor M.A. Viswanathan for his helpful advice.

The author also wishes to express her appreciation to Mr. Ismail Hashim for his help in much of the work and to Mrs. C.M. Ward for providing facilities in laboratory equipment for the physiological study.

The financial support from the Max Binz Scholarship is very gratefully acknowledged.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF PLATES	ix
Chapter	
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
III. MATERIAL AND METHODS	8
A. Sources of the Stilboid Fungi and Seeds of White Clover (<u>Trifolium repens</u>)	8
B. Methods for Studying the Conidia and the Morphology of the Stilboid Fungi	9
1. Observation of conidia	9
2. Germination of Conidia	9
3. Formation of Conidia	10
4. General Morphology of the Fungus ...	10
C. Methods for Studying Factors Affecting Growth and Sporulation of the Stilboid Fungi	11
1. Temperature	11
2. Media	12
3. pH of a Medium	14
4. Light	15
D. Method for Studying the Competition between the Stilboid Fungus and other Microorganisms	16
E. Method for Studying Morphological Abnormalities of the Stilboid Fungus Induced by Volatile Metabolites from Bacteria	18
F. Method for Studying the Survival in Soil	20

Table of Contents (cont'd)

Chapter	Page
III. G. Host Range Study Methods	21
1. Collection of Plants	21
2. Isolation Method	21
H. Host Parasite Relationship Studies	22
1. Host and Parasite in a Sterile Environment	22
2. Histological Methods	28
IV. EXPERIMENTAL RESULTS	32
A. Morphology of the Stilboid Fungi	32
B. Factors Affecting Growth and Sporulation of the Stilboid Fungi	58
1. Temperature	58
2. Media	68
3. pH of a Medium	95
4. Light	95
C. Competition between the Stilboid Fungus and other Fungi	103
D. Morphological Abnormalities of the Stilboid Fungus Induced by Volatile Microbial Metabolites	108
E. Survival in Soil	115
F. Host Range of the Stilboid Fungus	116
G. Host Parasite Relationship	123
1. External Appearance of White Clover Inoculated with the Stilboid Fungus...	123
2. Internal Appearance of Plants Infected by the Stilboid Fungus	125
V. DISCUSSION AND CONCLUSION	131
VI. SUMMARY	133
VII. REFERENCES	135

LIST OF TABLES

Table		Page
1.	The effect of temperature on growth and sporulation of the stilboid fungus on potato dextrose agar for 18 days in continuous darkness	56
2.	Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in continuous darkness and alternating periods of light and dark for 17 days at 25 degree centigrade	62
3.	Growth and sporulation of <u>Stilbum zacalloxanthum</u> Moore on seven kinds of nutrient media in continuous darkness and alternating periods of light and darkness for 17 days at 25 degree centigrade	65
4.	Growth rate of the single isolates of the stilboid fungus and <u>Stilbum zacalloxanthum</u> Moore on potato dextrose agar at room temperature with about twelve hours daily light period	86
5.	The effect of the initial pH of potato dextrose agar media on the growth of the stilboid fungus for 10 days at 20 degree centigrade	89
6.	The effects of continuous light, continuous darkness and alternating periods of light and dark on colony diameter and sporulation of single spore isolates of the stilboid fungus on potato dextrose agar for 18 days at 20 degree centigrade	91
7.	Growth rate of the stilboid fungus in continuous light, continuous darkness, and alternating periods of light and dark on potato dextrose agar at 20°	93

LIST OF FIGURES

Figure		Page
1.	Growth and sporulation of the stilboid fungus on potato dextrose agar for 18 days in continuous darkness at different temperatures	57
2.	Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in continuous darkness for 17 days at 25°	63
3.	Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°	64
4.	Growth and sporulation of <u>Stilbum zacallo-xanthum</u> Moore on seven kinds of nutrient media for 17 days in continuous darkness at 25°	66
5.	Growth and sporulation of <u>Stilbum zacallo-xanthum</u> Moore on seven kinds of nutrient media in alternating light and dark periods for 17 days at 25°	67
6.	Colony diameters of fungi grown on seven kinds of nutrient media in continuous darkness for 17 days at 25°	82
7.	Colony diameters of fungi grown on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°	83
8.	Sporulation of fungi grown on seven kinds of nutrient media in continuous darkness for 17 days at 25°	84
9.	Sporulation of fungi grown on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°	85
10.	Comparative daily growth rate of fungi from single spore isolates, on potato dextrose agar at room temperature for 13 days	87

List of Figures (cont'd)

Figure		Page
11.	Effect of the initial pH of potato dextrose agar on the growth of the stilboid fungus for 10 days at 20°	90
12.	Growth and sporulation of the stilboid fungus in continuous light, continuous dark, and alternating periods of light and dark on potato dextrose agar for 18 days at 20°	92
13.	Growth rate of the stilboid fungus in continuous light, continuous darkness, and alternating periods of light and dark on potato dextrose agar for 18 days at 20°	94

LIST OF PLATES

Plate		Page
1.	Stacked culture dishes of plants and plant parts in sterile water	23
2.	Clover plant growing with its roots in a sterile environment	26
3.	Anastomosed hyphae of the stilboid fungus, with conidiophores and conidia.	38
4.	Camera lucida drawings illustrating the types of conidiophores of the stilboid fungus	39
5.	Camera lucida drawings illustrating the stages of conidial formation by the stilboid fungus	40
6.	Conidial formation by the stilboid fungus	41
7.	Successive conidia forming a slime-ball	41
8.	Camera lucida drawings of macroconidia and microconidia of the stilboid fungus, conidiophore of the stilboid fungus arising directly from a germ tube; and conidia of <u>Stilbum zacalloxanthum</u> Moore	42
9.	Shape and internal features of conidia	43
10.	Camera lucida drawings illustrating conidial germination of the stilboid fungus	44
11.	Early stage of hyphae aggregations to form synnemata of the stilboid fungus	45
12.	The head of a synnema teased apart to show the production of conidia at the apices of the component hyphae	46
13.	Diagram showing features of synnemata	47
14.	Primary synnema growing from inoculated soil, producing a small secondary synnema from its head	48

List of Plates (cont'd)

Plate		Page
15.	Some hyphae of the synnematal stalks producing conidia	49
16.	Synnemata of the stilboid fungus growing on clover	50
17.	Synnemata of the stilboid fungus arising from stroma on potato dextrose agar	51
18.	Verticillated conidiophores of <u>Stilbum zacalloxanthum</u> Moore	52
19. and 20.	Clusters of conidia produced on verticillate conidiophores of <u>Stilbum zacalloxanthum</u> Moore	53
21.	Tiny droplets adhering to conidiophores and aerial hyphae of <u>Stilbum zacalloxanthum</u> Moore	54
22.	Agar cultures of the stilboid fungus and <u>Stilbum zacalloxanthum</u> Moore	55
23.	Cultures of the stilboid fungus grown in continuous darkness at 15, 20, and 28 degrees centigrade on potato dextrose agar for 18 days	60
24.	Cultures of the stilboid fungus grown at 10, 25, and 32 degrees centigrade on potato dextrose agar for 18 days in continuous darkness	61
25.	A culture of the stilboid fungus grown in continuous darkness on potato dextrose agar with alternating temperatures of 20° and 25° at 12 hour intervals for 18 days ...	61
26.	The growth and colony appearance of the stilboid fungus on seven kinds of media in continuous darkness at 25° for 17 days ..	77
27.	The growth and colony appearance of the stilboid fungus on seven kinds of media in alternating light-dark periods at 25° for 17 days	77

List of Plates (cont'd)

Plate		Page
28.	The culture of the stilboid fungus on czapek solution agar in alternating light-dark periods and in continuous darkness, for 17 days at 25°	78
29.	Zonation of the stilboid fungus	79
30. and 31.	Cultures of <u>S. zacalloxanthum</u> grown on various media in continuous darkness and alternating periods of light and dark for 17 days at 25°	80
32.	<u>Stilbum zacalloxanthum</u> Moore on potato dextrose agar showing the beginning of zonations made of spore laden synnemata	81
33.	Cultures of the stilboid fungus grown at 20° in continuous darkness, alternating periods of light and dark, and continuous light, on potato dextrose agar for 18 days ..	99
34.	A culture of the stilboid fungus grown at 20° in alternating light-dark periods, each of 24 hours duration on potato dextrose agar for 18 days	100
35.	Cultures of the stilboid fungus grown at 20° on potato dextrose agar for 22 days in alternating light-dark periods schedule of 5-days light - 5-min dark, and 5-days dark - 5-min light.	101
36.	Cultures of <u>Stilbum zacalloxanthum</u> Moore grown for 17 days at 25° on potato dextrose agar in alternating lightdark schedule of 12-hr light - 12-hr dark, and in continuous darkness	102
37.	The stilboid fungus growing with various fungi	105
38.	Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by <u>Agrobacterium tumefaciens</u>	111

List of Plates (cont'd)

Plate		Page
39.	Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by <u>Rhizobium leguminosarum</u>	112
40.	Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by <u>Bacillus cereus</u>	113
41.	Cultures of the stilboid fungus grown with <u>Agrobacterium tumefaciens</u> , <u>Bacillus cereus</u> , and <u>Rhizobium leguminosarum</u> , on nutrient agar for 7 days at room temperature	114
42.	7-day culture of the stilboid fungus on nutrient agar at room temperature	114
43.	The cultures of 21 isolates of the stilboid fungus obtained from various host plants	121b
44.	Five treatments of two-month old white clover with the stilboid fungus	127
45.	The root systems of white clover plants that had been treated with five inoculation methods	128
46.	Longitudinal section of white clover root infected with the stilboid fungus which is shown in the cortex	129
47.	Transverse section of an infected white clover root showing the stilboid fungus in the cortex intercellularly and intracellularly	129
48.	Longitudinal and transverse sections of inoculated white clover root showing the stilboid fungus in and between the cells of the cortex	130

I. INTRODUCTION

A stilboid fungus, first found by Estey (1960), was sent to the Mycology Section of the Canada Department of Agriculture, Ottawa, Canada, and to the Commonwealth Mycological Institute, Kew, England, for identification. Both agreed that it was an undescribed species.

A superficial examination of this fungus indicated that it is a member of the genus Stilbella Lindau, Family Stilbellaceae, Class Deuteromycetes. For some years there had been uncertainty and disagreement as to which name: Stilbella Lindau or Stilbum Tode ex Fr., more properly designates this genus, and whether Stilbellaceae or Stilbaceae should be used as the family name. Benjamin (1968) reviewed this nomenclatural problem and decided to conserve Stilbella Lindau and to validate Stilbellaceae. The author of this thesis agrees that these names should be accepted, for reasons given by Zalokar (1962) and reiterated by Benjamin (1968). The unnamed stilboid fungus of this study is therefore considered to be a new species of the genus Stilbella Lindau. Because it has not yet been given a name, it will simply be referred to, in this thesis, as the stilboid fungus.

A review of the literature concerned with the species of Stilbella Lindau or Stilbum Tode ex Fr., showed that only Stilbum zacalloxanthum Moore had features that were, in some respects, morphologically similar to the stilboid fungus under

study. Therefore, a subculture of S. zacalloxanthum Moore was obtained from the Commonwealth Mycological Institute to use for comparative purposes. Some of the morphological and physiological studies included in this thesis dealt with both stilboid fungi but most were done with the unnamed stilboid fungus alone.

II. REVIEW OF LITERATURE

The genus Stilbum was proposed in 1790 by Tode. Six described species were listed: S. vulgare; S. bulbosum; S. rubicundum; S. minimum; S. turbinatum; and S. pubidum.

In 1801, Persoon recognized 16 species, including S. vulgare Tode, which was validated by Merat and accepted by Fries, (Benjamin, 1968). Fries in his Systema Mycologicum, assigned Stilbum its own family, "Ordo Stilboidei" but included insufficient data to validate the genus. He recognized 22 species including S. vulgare, S. bulbosum, S. turbinatum, and S. pubidum of Tode and classified them in the Class Hyphomycetes. According to Benjamin (1968), Merat was the first post-starting date author to recognize Stilbum Tode. He validated it by providing a description of the genus and six species, including only one, S. vulgare, of Tode's species. From 1790 to 1898, Stilbum Tode was the accepted name for this Hyphomycete genus although it was classified differently by various workers. According to Ainsworth and Bisby (1954), 140 species had been described in Saccardo's Sylloge Fungorum between 1882 and 1931. In 1849, Fries proposed the family name "Sporocybaceae" based on the genus Sporocybe to replace "Stilbaceae" with the reason that: the family name Stilbaceae is used for the genetic type Stilbe. He maintained Stilbum as a member of Sporobaceae, but this proposed change gained little acceptance. The name Sporocybaceae would not be tenable for the present Stilbaceous

4

family since Mason and Ellis (1953) have shown that Sporocybe Fr. is the synonym of Periconia Tode ex Fr. In 1886, Saccardo reduced the genus Botryonipha Preuss to a subgenus of Stilbum Tode. Then Kuntze in 1891, replaced Stilbum Tode with Botryonipha Preuss. He stated that the generic names Stilbe Berg. (1767) and Stilbum Tode (1790) were orthographic variants, but Kuntze's change seems to be unnecessary according to Article 75 of the International Code of Botanical Nomenclature (Lanjouw, 1961).

Stilbum Tode remained a widely accepted name for an imperfect genus until 1898, when Juel discovered that Stilbum vulgare was really a Basidiomycete. He transferred this species and the genus to the Auriculariaceae with S. vulgare as the type genus and left the remaining imperfect species without generic name. Lindau in 1900 erected the name Stilbella to replace that of Stilbum as the genus of fungi imperfecti. He did not designate a new type species, but did transfer from Stilbum the following species: Stilbella hirsutum (Hoffm.) Lindau; S. erythrocephala (Ditm.) Lindau; S. bulbosum (Tode) Lindau; S. cinnabarinum (Mont.) Lindau; S. lejopus (Ehrenb.) Lindau; and S. dubium (Preuss) Lindau. He maintained Botryonipha Preuss as a subgenus. According to Article 63 of the Code, Stilbella is an illegitimate name since Botryonipha was an available synonym at that time. Saccardo in 1902, maintained Stilbum as an interim genus of the fungi imperfecti. In this case the name would be cited

as Stilbum Sacc. & Syc., instead of Tode per Merat, under Article 48 of the Code (Lanjouw, 1961). Clements and Shear (1931) designated Stilbum cinnabarinum as the type species for Stilbum Tode; and Stilbella erythrocephala as the type for Stilbella Lindau. Stilbum cinnabarinum could not serve as a lectotype species since it does not represent the original material as required under Article 7 of the Code. Stilbella erythrocephala would seem appropriate as the lectotype species if the genus is conserved.

Martin (1940) recognized Stilbella Lindau and proposed Stilbellaceae to replace Stilbaceae for the imperfect genus. but there was insufficient data for valid publication as required by Article 32 of the Code. In the past, the use of the generic name Stilbum or Stilbella and the family name Stilbaceae or Stilbellaceae have been in a state of confusion and disagreement among the workers. Wakefield and Bisby (1941), Mains (1948), and Moore (1959), recognized the generic problem but preferred to use the name Stilbum with their different reasons. Barnett (1960) also used Stilbum but accepted S. cinnabarinum as a lectotype species. Hughes (1958) used Stilbum but cited it as Stilbum Pers. and designated S. rigidum as a new type species as he proposed a change of starting point for names of the Hyphomycetes from Fries' Systema to Persoon's Synopsis and not citing pre-1801 authorities (Hughes, 1957). Donk (1963) and Benjamin (1968) disagree with Hughes's change of starting point because the Code does not authorize

one to change the starting point of Hyphomycetes and brush away all earlier typification of the name as well, even though the other lectotype had been designated. Donk (1963) also proposed that the corrected generic name should read Stilbum Tode per Merat, not Stilbum Tode per Fr., or Stilbum Pers.

Morris (1963) recognized Stilbellaceae Martin and Stilbella Lindau. He listed Botryonipha Preuss and Cephalopharum Nees as generic synonyms in addition to Stilbum Auct. (not Tode ex Fr. sensu Lindau). According to Benjamin (1968), both names should have been listed as doubtful synonyms. Stilbella and Stilbellaceae are increasingly being accepted by recent workers, including Gilman (1945 & 1957); Bessey (1950); Martin (1961); Alexopoulos (1962); and Zalokar (1962). Zalokar (1962) accepted Stilbum as the valid name of the Basidiomycete genus and Stilbella as the valid name for the Hyphomycete genus. He transferred all the species previously assigned to the genus Stilbum Tode ex Fr. to the genus Stilbella Lindau as a new combination. Additionally, he gave a complete monograph of the genus including a key to 28 species. Benjamin (1968) solved the generic problem by conserving Stilbella Lindau with S. erythrocephala as a lectotype species, and validated Stilbellaceae Martin with information required by the International Code of Botanical Nomenclature (Lanjouw, 1961).

Stilbella Lindau is a large genus. Species of the genus are commonly found as entomogenous fungi, (Smith, 1903);

Wakefield & Bisby (1941); Petch (1945); Mains (1948). Stilbum-like fungi are not uncommon in soil (Barron, 1968). They are also found on stems of dicotyledon plants, on wood and bark (Barnett, 1960; and Sutton, 1973); on oak leaves, on rotten branches, on fungi and on various animals dung (Wakefield & Bisby, 1941). Zalokar (1962) listed 188 species including 28 species which he had studied. In addition, Moore (1959) found Stilbella zacalloxanthum (Stilbum zacalloxanthum Moore) in 1959, and Fergus (1964) found Stilbella thermophila from mushroom compost. S. thermophila seems to be the only thermophilic species and the latest species to have been reported in the literature.

III. MATERIAL AND METHODS

A. SOURCES OF THE STILBOID FUNGI AND SEEDS OF WHITE CLOVER, TRIFOLIUM REPENS L.

The initial culture of the unnamed stilboid fungus was isolated from white clover, Trifolium repens, which had been growing in the lawn at the left wing of the Biology Building of Macdonald College, Ste Anne de Bellevue, Quebec, Canada. The clover plant with its root system was gently washed in running tap water and then immersed in sterile water in Petri dish. Some of the leaves were removed. Synnemata of the fungus appeared from various parts of the clover in 7 to 15 days. Spores were transferred from the head of the synnemata to the surface of potato dextrose agar (PDA) in Petri dishes by means of an inoculating needle. The fungi that grew from these spores were subcultured on additional PDA in dishes and culture tubes for subsequent study. Stock cultures, on culture slants, were kept in a cold room.

The culture of Stilbum zacalloxanthum Moore, IMI 79934, was bought from the Commonwealth Mycological Institute, Kew, Surrey, England.

The seeds of Trifolium repens were obtained from Dr. N. Lawson of the Agronomy Department, Macdonald College.

B. METHODS FOR STUDYING THE CONIDIA AND THE MORPHOLOGY OF THE STILBOID FUNGI

1. Observation of Conidia

The conidia obtained from the head of the synnemata of fungi growing on host plants or culture media, were mounted in either water, lactophenol, or cotton blue in lactophenol on a glass slide. The slide mounts were then examined with a compound microscope under low and high magnifications.

The size of the conidia was determined by means of a calibrated filar micrometer. The determined size of the conidia is the average of the measurements of 100 conidia.

2. Germination of Conidia

The dilution plate method (Anon, 1968) was employed to separate conidia for germination studies. Conidial suspensions in culture tubes were diluted with sterile water so that when one millilitre of the resulting suspension was transferred to the surface of a thin film of potato dextrose agar in a 9 cm Petri dish the conidia were widely separated from each other.

Germination was observed after 10 to 30 hours, under the compound microscope. A cover glass was placed over a selected conidium and the Petri dish was fixed to the stage of the microscope with adhesive tape. When changing the objectives of the microscope, care was taken so as not to move the dish, thus the same conidium could be observed at

different magnifications over a period of time. The stages of germ tube development were observed, and drawn with the aid of a camera lucida attachment to the microscope.

3. Formation of Conidia

Observation of conidial formation was usually done about two days after germ tube production, and the same method as for the observation of conidia germination.

4. General Morphology of the Fungus

The general morphology of the fungus was observed as the fungus grew on slide cultures (Anon, 1968). A small (about 7 mm square) block of PDA cut from a Petri dish was placed on a microscope slide. It was inoculated with a number of spores, then covered with a cover glass and incubated in a Petri dish containing sterile moist filter paper to maintain a humid environment. About 3 to 4 days later, when the culture was mature, some sporing structures would adhere to the slide and cover glass. The agar block was then discarded and both the slide and the cover glass were used to make mounts of the fungus in either lactophenol, or cotton blue in lactophenol.

C. METHODS FOR STUDYING FACTORS AFFECTING GROWTH AND SPORULATION OF THE STILBOID FUNGI

1 . Temperature

The fungi were cultured under different temperature conditions. The temperatures used in this study were; 10, 15, 20, 25, 28, 32, and 40 degrees centigrade. Difco PDA was prepared according to the directions on the label and 15 ml of this medium was poured in each Petri dish. Inoculum of uniform size was obtained by using a sterile cork borer, 4 mm in diameter to cut out small discs of mycelium from the periphery of a culture of the fungus that had grown for 15 days on an agar medium. Each disc of inoculum was transferred to the center of the PDA in Petri dishes by means of an inoculating needle.

The Petri dishes with the inocula were wrapped with aluminium foil and placed in cabinets having 10, 15, 20, 25, 28, 32, and 40 degrees centigrade controlled temperatures respectively. Five dishes were placed in each cabinet (plus a few extra dishes to substitute for any culture that became contaminated). The growth of the fungus was determined after 18 days by measuring the diameter of the colonies.

Sporulation was determined by cutting out the entire colony and placing it in a measured volume of water in a 125 ml Erlenmeyer flask. The flasks were shaken for at least 30 min. and spore counts were made by using a haemocytometer.

The stilboid fungus cultures were used for testing

the effect of changing of temperature to the growth rhythm of the fungus. They were kept in a plant growth chamber which was adjusted to have a temperature schedule of 12 hours at 20° followed by 12 hours at 25° without light. Growth rhythms were observed after 18 days.

2. Media

a. Growth of the Stilboid Fungus on Different Media

Nutrient media used in this study were from Difco Laboratories and were prepared according to the directions on the labels. There were seven kinds of media, the specifications of which are:

Media	Formula	pH
Cornmeal agar	Infusion of corn meal Bacto-agar	5.6
Czapek solution agar	Saccharose Sodium nitrate Dipotassium phosphate Magnesium sulphate Potassium chloride Ferrous sulphate Bacto-agar	7.3
Lima bean agar	Infusion of lima bean Bacto-agar	5.6
Malt extract agar	Infusion of malt Bacto-agar	4.6
Mycological agar	Bacto-yeastone Bacto-dextrose Bacto-agar	7.0
Nutrient agar	Celysate peptone Beef extract Bacto-agar	6.8

Media	Formula	pH
Potato dextrose agar	Infusion of potato Bacto-dextrose Bacto-agar	5.6

About 15 ml of these media was poured into each Petri dish in which fungi were to be cultured. One set of inoculated culture dishes was left unwrapped during the growing period and another set was wrapped in aluminium foil; both sets were placed in polyethylene bags. For incubation all cultures were placed in the same plant growth chamber the temperature of which was adjusted to 25° with the light schedule of 12-hr light - 12-hr dark intervals.

The effect of the nutrient media on the growth characteristics of the colonies and their sporulation was determined after 17 days. Growth was measured by the diameter of the colonies and sporulation was determined by the total spore-count, as in (1) above.

B. Growth Rate of the Stilboid Fungi

This experiment was done to find the comparative growth rates of the unnamed stilboid fungus and Stilbum zaealloxanthum. The nutrient medium used was potato dextrose agar from Difco Laboratories. Single spores were obtained by the dilution plate method as described above. One spore was transferred into the center of each of the PDA plates by means of an inoculating needle. Four cultures of each fungus, from spores that had germinated at about the same time, were used in this

test. All cultures were kept under the same environmental conditions at room temperature (about 25°). The diameter of the colonies was measured every 24 hours for 13 days. The results of these measurements are given in Table 4.

3. pH of a Medium

The nutrient medium used in this experiment was PDA from Difco Laboratories, prepared according to the directions on label. About 100 ml of this medium was poured into each of eleven 250 ml Erlenmeyer flasks, which were then sterilized at 120° under 15 lb. pressure, for 15 minutes. When the medium had cooled to about 40°, each of these flasks was adjusted to have a pH of 2.5, 3.0, 4.0, 4.5, 5.6, 6.5, 8.0, 9.0, 10.5, 11.0, and 11.5. The acid and basic ranges were adjusted by using normal hydrochloric acid and normal sodium hydroxide respectively. The pH levels were determined by means of a glass electrode potentiometer. The medium from each of these flasks was then poured into each of four Petri dishes, and inoculated with a 4 mm disc of the stilboid fungus. The cultures were wrapped with aluminium foil and were kept in a cabinet with controlled temperature at 20°. Growth of the fungus at each pH level was determined by measuring the diameter of each colony after 10 days. These measurements are given in table 5.

4.. Light

(a) Light experiments were carried out in two plant growth chambers illuminated by a bank of fluorescent lamps having 15,000 ft-candles of light intensity. The temperature of the cabinets was set at 20°. The fungus was cultured in Petri dishes containing PDA from Difco Laboratories. Five dishes were used for each of these treatments, which were:

- i. Continuous light.
- ii. Continuous darkness.
- iii. Alternating light and dark periods.

The cultures of treatment (i) and (ii) were kept in the same cabinet, but the cultures grown in the dark were wrapped in aluminium foil and placed in light proof boxes.

The cultures of treatment (iii) were kept in another plant growth chamber which was set to have light for 12 hours and dark for 12 hours alternately.

The effect of light on the growth of the fungus was determined after 18 days by measuring the diameter of colony growth. Sporulation was determined by the total spore count method.

(b) The result in the first light experiment (a) showed that a growth rhythm of the stilboid fungus was stimulated by light. In order to see more of this effect, the following experiment was carried out to show the effects

of light on the zonation of the fungus.

The fungus was inoculated on PDA plates as in (a) and kept in the same cabinet with alternating light-dark periods as follow:

- i. 24 hours light and 24 hours dark interval.
- ii. 5 days light and 5 minutes dark interval.
- iii. 5 minutes light and 5 days dark interval.

In the light period, the cultures were exposed to light intensity of 15, 000 ft-candles. The dark period was achieved by wrapping the dishes in aluminium foil. The cultures were observed after an appropriate number of light and dark cycles.

D. METHOD FOR STUDYING THE COMPETITION BETWEEN THE STILBOID FUNGUS AND OTHER MICROORGANISMS

Twenty species of fungi obtained from the type culture collection of the Plant Pathology Department of Macdonald College were grown to compete with the stilboid fungus. The experiment was done in Petri dishes containing PDA. Each fungus was inoculated side by side with, but about 50 mm away from, the stilboid fungus. The 20 species of fungi used were:

1. Alternaria sp.
2. Arthrobotrys superba
3. Aspergillus repens

4. Botrytis sp.
5. Chaetomium sp.
6. Diplodia zeae
7. Fusarium oxysporum
8. Gliocladium roseum
9. Gliomastrix sp.
10. Helminthosporium sativum
11. Mucor hiemalis
12. Oospora sp.
13. Penicillium expansum
14. Phoma sp.
15. Sclerotium rolfsii
16. Sordaria sp.
17. Stachybotrys atra
18. Stilbum zacalloxanthum
19. Tricoderma sp.
20. Verticillium dahliae

For each fungus, the inoculum consisted of a 4 mm disc cut from the periphery of a young culture. Inoculation was done as follows:

1. One of the above fungi was inoculated to a point from the periphery of the medium that was equal to about one third of the diameter of the dish. When its growth had reached a diameter of 3 centimeters, the stilboid fungus was inoculated to a similar point directly across from the first fungus.

2. The stilboid fungus was inoculated first followed by the other fungus. This is the reverse of (1) above.

3. The stilboid fungus and one of the other fungi were inoculated to the culture medium at the same time.

In each case the cultures were kept at room temperature until the two colonies met and/or showed reactions at the region of contact.

E. METHOD FOR STUDYING MORPHOLOGICAL ABNORMALITIES
OF THE STILBOID FUNGUS INDUCED BY VOLATILE
METABOLITES FROM BACTERIA

In this experiment, the stilboid fungus was exposed to the volatile metabolites produced by the bacteria; Agrobacterium tumefaciens, Bacillus cereus, and Rhizobium leguminosarum.

(1) The bacteria were cultured on Difco nutrient agar in Petri dishes and on nutrient broth in 250 ml Erlenmeyer flasks. The stilboid fungus was cultured on Difco PDA. The bacteria were grown on the nutrient agar either as film cultures or as streak cultures. The film cultures were produced by transferring 1 ml of a 24-hour broth culture of bacteria to nutrient agar plates using a sterile pipette and then spreading it to produce an even film by rotating the plates. Streak cultures were obtained by streaking a loopful (about 0.01 ml) of the 24-hour broth culture on the nutrient

agar by using a single calibrated looped inoculating needle. Streak cultures produced a much lower bacterial population than did the film cultures.

By using a sterile razor blade, a block of PDA (about 7 x 7 mm) was cut out and placed on a sterile microscope slide. The stilboid fungus was inoculated at the four corners of this block. Five of these culture slides were prepared for each bacterium used; 2 for streak cultures, 2 for film cultures, and one for control. They were then placed inverted on a bent glass rod (5 mm in diameter) in Petri dishes containing either a film culture or a streak culture freshly inoculated with bacteria so that the slide culture of the stilboid fungus was facing down to the bacterium culture. Control cultures were grown by the same method as described above but were set over sterile nutrient agar only.

All cultures were kept at room temperature in approximately a 12 hour daily light period. The morphological abnormalities of the stilboid fungus were examined after 6 to 10 days. Whole mount slides of the stilboid fungus were prepared by removing the agar block and immersing the slide in 70% alcohol. The alcohol was drawn off and 4% potassium hydroxide was then added to bring the specimen back to its original size. The slides were finally mounted in either water, lactophenol, cotton blue in lactophenol, or 1% acid fuchsin in lactic acid for wall staining. Drawings were made with the aid of a camera lucida apparatus.

(2) Each of the three species of bacteria were grown in the same culture dish, of nutrient agar, with the stilboid fungus. The inocula of bacteria were streaked on the agar medium by means of a calibrated looped inoculating needle. The fungus inoculum was a 4 mm disc of mycelium that had been growing on PDA. Both the bacteria and the stilboid fungus were inoculated side by side in the nutrient agar dishes at the same time. They were then kept at room temperature and exposed to approximately 12 hours of daily light and dark periods. The growth of both colonies was examined when they met and/or showed reaction in the region of contact.

F. METHOD FOR STUDYING THE SURVIVAL IN SOIL OF THE STILBOID FUNGUS

Sterilized soil was placed in ten pots which were placed on a bench in the greenhouse. After 10 days the soil was inoculated with 10 ml of a suspension having 2×10^6 conidia per ml of the stilboid fungus. The soil was kept moist by the addition of water whenever necessary. After 14 days the soil was transferred to sterile 9 cm Petri dishes. The subsequent appearance of the synnemata characteristic of fungus was observed under a dissecting microscope.

Natural soil was collected from the lawns of the Macdonald Campus during the summer of 1973. Small amounts of this soil were placed in sterile Petri dishes. When necessary, water was added to moisten the soil. The dishes

were put into polyethylene bags to retain moisture and then placed on a laboratory shelf until the stilboid fungus was observed.

G. HOST RANGE STUDY METHODS

1. Collection of Plants

The areas from which plants were collected for this study included the Macdonald College Campus, and the townships of Ste Anne de Bellevue and Dorion (4 miles from Ste Anne de Bellevue), Quebec, Canada. The plants growing in these areas were collected after first digging around the root system with a trowel to loosen the soil. In the case of Acer saccharum, seedlings and bark were collected. Each plant specimen with its intact roots was put in a separate polyethylene bag. Collections were made from April to October, 1973. There were altogether 60 plant specimens, as listed on page 118.

2. Isolation Method

The plants were thoroughly washed in running tap water, then they were trimmed or divided into root, stem and leaf parts that would fit into the culture dishes. After being placed in the culture dishes just enough sterile water was added to flood the root or other part of the plant from which the fungus was expected to be isolated. The dishes

were stacked, as illustrated in Plate 1, and left on a laboratory shelf at ordinary room temperature and daily light periods. Fungal isolations were made when synnemata appeared which were visible to the naked eyes. Spore isolates, taken from the heads of the synnemata, were transferred to plates of PDA. Each isolate was then transferred to tubed slants of PDA and stored in a room at 5°.

Some of the standard methods of isolating fungi from plant material were tried but only the above method gave consistently successful results.

H. HOST PARASITE RELATIONSHIP STUDIES

1. Host and Parasite in a Sterile Environment

a. Planting in Bottles

The plant used for this experiment was white clover (Trifolium repens L.). The clover seeds were surface sterilized by immersion in 10% Javex for 10 to 15 minutes. Five of the surface sterile seeds were then spaced on dilute PDA in each of several Petri dishes. The Difco PDA was diluted with double the normal amount of water. Germination usually occurred after 2 days. When the seedlings were about 10 to 20 days old, those that were shown to be free of microorganisms were transplanted into glass jars previously prepared as follows:

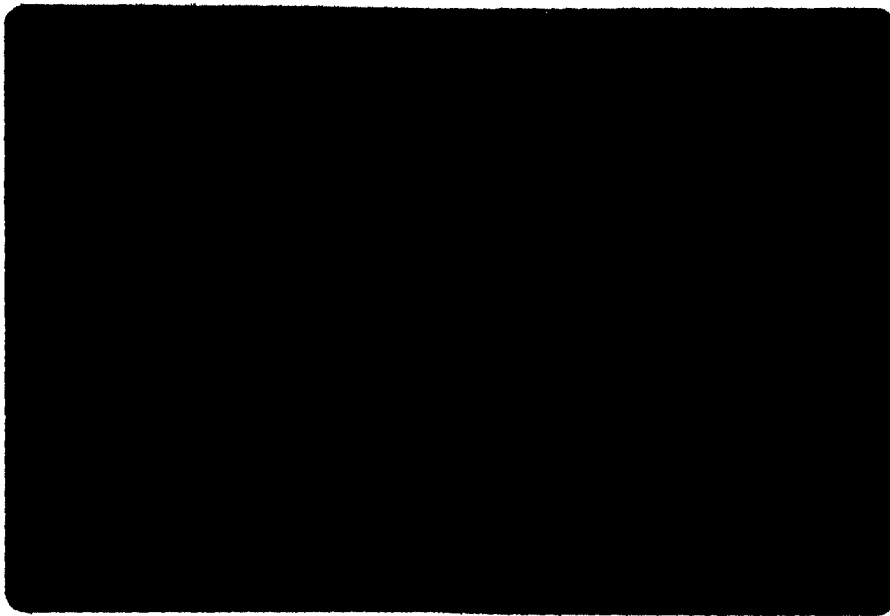


Plate 1. Stacked culture dishes of plants and plant parts
in sterile water.

The jars were filled with a mixture of moistened soil and perlite. They were then stoppered with a 3-holed cork stopper carrying one bent glass tube in each of two holes, thus leaving the third hole to be plugged with cotton. The end outside the bottle of one of the glass tubes was plugged with cotton while a rubber stopper was used to plug the corresponding end of the other. The rubber stoppered tube was used for adding fluids to the bottle by means of a hypodermic needle. The bottles were then twice sterilized at 120° under 15 lb pressure for 45 minutes each. Sterilized melted wax was then poured through one of the tubes to seal the space between the soil mixture and the stopper as well as the spaces around the glass tubes. The bottles were left to cool until the seedling plants were ready to be transplanted.

Seedling transplantation to the bottles was done under the aseptic conditions of a transfer chamber. The cotton plug sealing the hole of the cork was removed and a sterilized needle was inserted into the hole to pierce a hole through the layer of the wax. The root system of a seedling was then inserted into the hole so that the roots were in contact with the soil below. The space around the plant in the hole was then sealed with petrolatum, a soft mixture of petroleum jelly and paraffin wax. The transplanted seedlings were kept in a plant growth chamber adjusted to have 10 hours of darkness followed by 14 hours of light at an intensity of 15,000 ft-candles, at 25° . Sterile water was syringed into

the bottle whenever the soil appeared to be getting dry. One such glass jar, containing a six-week old white clover plant, is shown in Plate 2. After the seedlings produced four to five leaves, each plant was inoculated with 10 ml of a conidial suspension of the stilboid fungus having a concentration of 4×10^6 conidia per ml. The control plants in other bottles received only sterile water. The plants were removed from the bottles 14 days after inoculation. Fungus isolation was aseptically made to check for the presence of the stilboid fungus or other contaminants. Portions of the clover plants suspected of being infected with the stilboid fungus were fixed in formalin acetic acid for later sectioning and staining.

b. Planting in pots of soil

Surface sterile white clover seeds were germinated and seedlings were grown for 15 days on PDA in Petri dishes as described above. The seedlings were then transplanted to steam sterilized soil in 10 cm clay pots. These seedlings were then kept in the same plant growth chamber under the same environmental conditions as the seedlings in glass jars, described above. Tap water was used to water the plants every day. Five treatments, consisting of 2 plants per treatment were made as follows:

1. Control

In this "treatment" the plants were not inoculated. They received only sterile water.

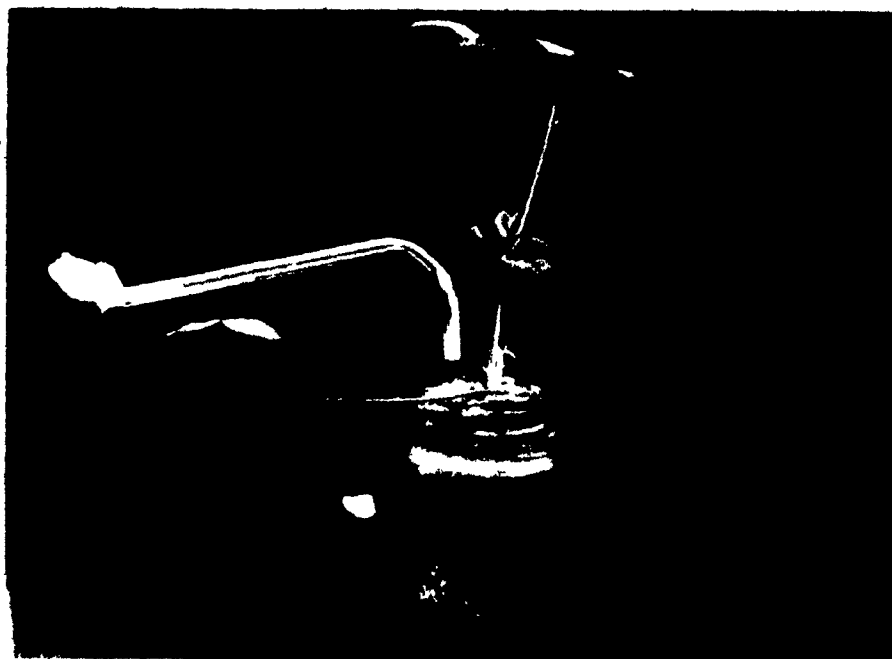


Plate 2. Clover plant growing with its roots in a sterile environment.

ii. Leaf inoculation

The leaves were mechanically wounded and then inoculated with fungus conidia by means of an inoculating needle.

iii. Petiole inoculation

This was done by the same method as for leaf inoculation except that the conidia were inoculated into the petioles.

iv. Root dip inoculation

The plants were uprooted and their root systems, which had a few wounds, were dipped in a suspension of conidia for about 2 to 5 minutes. They were then replanted in moist sterile soil.

v. Soil inoculation

A suspension of conidia was poured into the soil in which each plant was growing.

The concentration of conidia in the above mentioned suspension was 2×10^6 conidia per ml. All inoculations were performed when the plants were at the 5-leave stage or about five weeks old. After inoculation, the plants were kept under humid conditions by covering each plant with a polyethylene bag containing some pieces of wet cotton, to provide moisture inside the bags. The bags were removed after three days. About 14 days after inoculation, the plants were examined by uprooting and washing in tap water. Portions of the plants in each treatment were cut into small pieces which

were then fixed in formalin acetic acid.

2. Histological Methods

After the plant material had been fixed in formalin acetic acid for at least 48 hours, it was washed with two changes of 50% ethyl alcohol, dehydrated in a butanol series and embedded in paraffin wax (Sass, 1968). Sections were cut with a rotary microtome and mounted on glass slides. Stains used to stain the sections were: Pianese IIIb (Simmons & Shoemaker, 1952); carbol thionin-orange G (Gurr, 1956); Heidenhain's hematoxylin (Conn, 1960); and safranin-fast green (Sass, 1968). The staining procedures were as follows:

Pianese IIIb (Simmons & Shoemaker, 1952)

Solutions used: Pianese IIIb, which is:

Malachite green	0.50 g
Acid fuchsin	0.10 g
Martius yellow	0.01 g
Distilled water	150.00 ml
95% ethyl alcohol	50.00 ml

Method:

The sections were passed through three changes of xylene to remove paraffin.

Passed through descending grades of alcohol down to 50%

Stained in Pianese IIIb for at least 45 minutes.

Passed through two changes of absolute alcohol followed by acid alcohol (95% alcohol plus a few drops of HCl) for one half to one minute.

Passed through absolute alcohol.

Cleared in carbol xylene.

Mounted in Canada balsam.

Carbol Thionin - Orange G (Gurr, 1956)

Solutions used: Saturated carbol thionin solution.

Orange G, 0.2% in absolute alcohol.

Method:

The sections were passed through xylol and descending grades of alcohol down to distilled water.

They were stained for at least an hour in the carbol thionin solution.

Excess stain was poured off and the sections were rinsed with 70% alcohol followed by 90%.

Rinsed quickly in two changes of absolute alcohol.

To get differential staining the sections were placed in the orange G solution for varying periods of time, depending upon the desired intensity of stains.

They were then passed through 90% followed by 70% alcohol and rinsed well in water.

Then rinsed with 70% alcohol, followed by 90%.

Rinsed quickly in two changes of absolute alcohol.

Finally, they were passed through two changes of xylol, and were mounted in Canada balsam.

Heidenhain's Hematoxylin (Conn, 1960)

Solutions used: 3% aqueous ferric alum.

0.5% aqueous Heidenhain's hematoxylin.

Method:

The paraffin was removed from the sections with xylene and then they were passed through descending grades of alcohol down to water.

They were mordanted in aqueous ferric alum for 4 hours.

Then washed for 5 minutes in tap water, and rinsed in distilled water.

Stained for an hour in aqueous Heidenhain's hematoxylin.

Then they were washed for 30 minutes in running tap water.

Differentiated in aqueous ferric alum, controlling the differentiation by microscopic examination.

Then washed for 30 minutes in running tap water.

Dehydrated in ascending grades of alcohol.

Passed through three changes of xylene, and

finally mounted in Canada balsam.

Safranin and Fast Green (Sass, 1968)

Solutions used: 2% Safranin (aqueous).

1% Fast green in absolute alcohol.

Method:

The sections were passed through xylene and descending grades of ethyl alcohol down to distilled water.

Stained for 2 hours in 2% aqueous safranin.

Washed in three changes of water.

Brought up to 95% alcohol.

Stained for 30 seconds in the fast green solution.

Passed through three changes of absolute alcohol.

Cleared in carbol-xylene.

Passed through three changes of xylene.

Mounted in Canada balsam.

IV. EXPERIMENTAL RESULTS

A. MORPHOLOGY OF THE STILBROID FUNGI

The thalli of the stilboid fungus are bounded by true cell walls. The hyphae are hyaline and branch alternately. The average width of the hyphae is about 1.6 micrometers. It is septate and the average cell length is approximately 45 micrometers. The apical growth of the leader hyphae often bring them in contact with other hyphae growing nearby. They may anastomose with the same or different hyphae or just make contact and then separate. Examples of anastomosed hyphae are shown in Plate 3.

The conidiophores are composed of simple or branched hyphae which are not distinctively different from the somatic hyphae in size or general appearance (Plates 4, 6, and 7). They may be septate or non-septate (Plate 4 a and d). In length, they may be very short or very long but they are usually from 20 to 80 micrometers long. The shortest conidiophores appear as knob-like structures arising from the somatic hyphae (Plate 4 c and e). The final branches of somatic hyphae sometimes function as conidiophores (Plate 4 b). The elongation of the conidiophores and the initiation of conidia are confined to the apical regions of the conidiophores. The lateral branches of the conidiophores are usually formed singly but it is not uncommon to find a whorl of three arising at the base of the terminal sporogenous cell.

Conidia are born singly as blown out terminal ends of the conidiophores. The stages of conidial formation are illustrated in Plate 5. Each conidium of the observed culture was produced in approximately 130 minutes. This kind of conidium may be called an aleuriospore, according to Tubaki (1966). It is similar to Section III of Hughes (1953) classification, except that there is no evidence of the annellated conidiophore due to the scar left by the previous spore while the sporophore increases in length. The mature conidia usually fall or are pushed to one side of the conidiophore as a new one is being formed. Conidia of different sizes may be produced from the same conidiophore (Plate 4 d). These successively formed conidia usually adhere together in a slime-ball which may be pale to bright yellow in colour (Plate 7). A single conidium appears hyaline under the microscope. Conidia of varying sizes produced by a single colony of the fungus may be arbitrarily classed as macroconidia and microconidia, depending mostly upon their length (Plate 8 a and b). Macroconidia are considered to be those which are 1.5(-6.4 wide) x 11.5(-18.0 long) micrometers while those that are referred to as microconidia are 1.5(-5.7 wide) x 3.5(-9.5 long) micrometers. The conidia are unicellular and guttulated (Plate 9 g). Guttulation usually appears as one or two drops in microconidia whereas it was not uncommon for macroconidia to contain several of the drop-like structures. The cytoplasm stained blue around

the guttulations when cotton-blue in lactophenol was applied, thus making the conidia appear to be septate. The shape of the microconidia varies from ovoid, ellipsoid, to oblong and occasionally globose (Plate 9 a,b,c, and d). The macroconidia are mostly cylindrical or fusoid (Plate 9 e and f). The conidia germinate by means of one or more germ tubes. In a drop of water, the mature conidia will germinate in 10 to 30 hours after being removed from the conidiophore. The cell wall of a conidium protrudes as a germ tube initial. This may occur at the end or at any weakened area of the conidial wall. The germ tube then elongates into a typical hypha. Other germ tubes may be initiated, following the first one, at various places on the conidial wall. The second germ tube was usually formed about 23 hours after the first one. As many as four germ tubes have been seen protruding from one conidium, which may also give rise to a conidiophore directly after germination (Plate 8 d). Stages of germ tube development are shown in Plate 10. Chlamydospores are sometimes formed, the average size of which are about 5 x 20 micrometers. They are commonly bicellular with thickened walls.

Typically, the conidiophores of this fungus aggregate to form erect, columnar synnemata with sporogenous cells at their apices (Plate 11). The growth of a synnema is usually determinate, although it is not uncommon to see one or more hyphae growing through the spore bearing head of a synnema to form secondary synnema-like fruiting structures. The

component hyphae of a synnema may be simple, or they may be branched laterally or terminally (Plate 13 a,e, and f; and Plate 14). The column or stalk of a synnema is usually smooth but sometimes the hyphae of the synnema produced conidia (Plate 13 c, and Plate 15). Synnemata are produced singly or in groups of 3 to 5 or more (Plate 13 b, and d). The length of the synnemata, measured from their base to the top of the slimy heads, varies from 270 to 2,000 or more micrometers. The diameter of the mucoid heads vary from 59 to 330 or more micrometers. The width of the columns that support the heads are also quite variable, depending upon the number of conidiophores of which they are composed. Most of them are from 12 to 45 micrometers in diameter. The synnemata seen on host plants (Plate 16) were similar to those that arose on nutrient media (Plate 17). The variation in the size of the synnemata is probably due to differences in the nutritional status of the media on which the fungus is growing, the number of them that are produced per unit area of the substratum and to certain environmental factors such as the presence or absence of light and its quality or intensity. The developing synnemata were found to be positively phototropic, and illumination influenced their formation and their production of conidia. This latter factor will be dealt with in more detail later.

The morphological studies of Stilbum zacalloxanthum by the present author was done with cultures that were grown on potato dextrose agar. The submerged hyphae of this fungus

are hyaline while the aerial hyphae appear blackish in colour. The conidiophores are produced superficially. They are verticillate with a whorl of five or more branches (Plate 18). The conidia are produced singly at the apex of the conidiophores where they adhere in slime-balls. Plate 19 and 21 show the slime-ball as it appeared from top view of a culture. So many tiny droplets commonly form on the aerial hyphae as to give them a roughened appearance. An example of this is shown in Plate 21. They probably are exudates secreted by the fungus, or simply droplets of water, for they slowly evaporate when exposed to drying conditions. The conidia of this fungus are oblong (Plate 8 c), and smaller in size than those of the unnamed stilboid fungus (Plate 8 a and b). The cultures grown on PDA were of a brighter yellow than the stilboid fungus. Zonation occurred in the cultures of both fungi, corresponding to the alternating light-dark periods, but the margins of the S. zacalloxanthum cultures were more wavy or irregular than those of the unnamed stilboid fungus (Plate 22). The synnemata composed of aggregated hyphae or conidiophores, are morphologically similar in both species, but those of S. zacalloxanthum were generally shorter and otherwise smaller, and the conidia were much smaller, than the ones produced by the unnamed stilboid fungus under similar conditions.

According to Moore (1959), the synnemata of Stilbum zacalloxanthum are bright yellow and up to 0.75 mm high.

They are cylindrical to spathe-like, single or in groups of two or three, bearing terminally on increasingly large mucus drops up to 325 micrometers in diameter, replete with conidia. The conidia are bacilliiform, at maturity 1.5×3.0 (-7.5) micrometers. They are hyaline, borne monacrogenously on a slender sterigmata from cylindriform conidiophores.

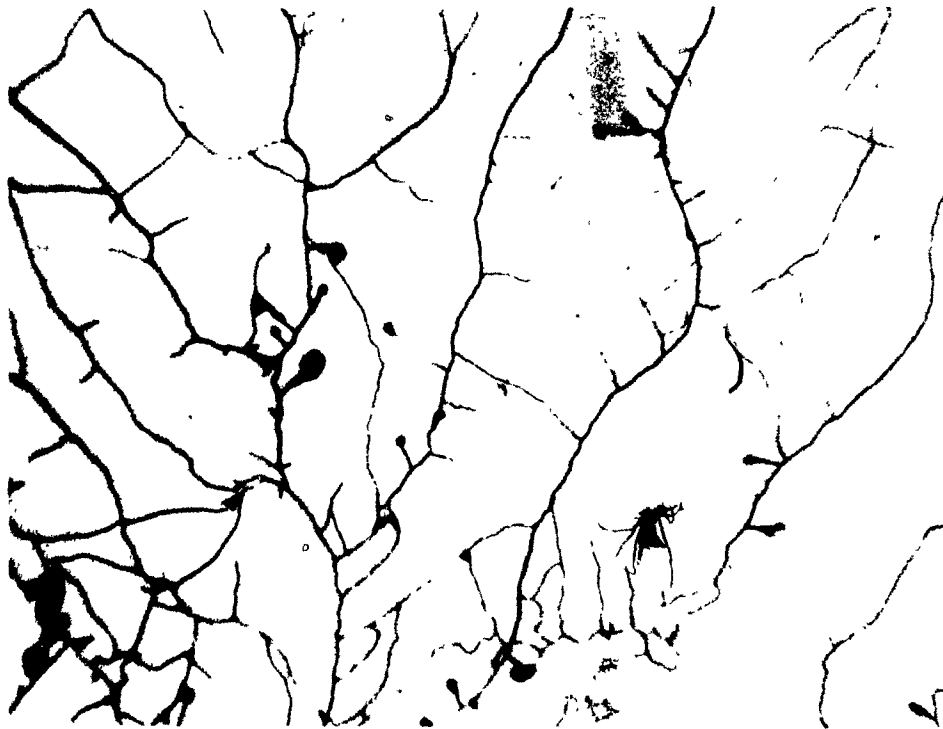


Plate 3. Anastomosed hyphae of the stilboid fungus, with conidiophores and conidia. Mag. 300X.

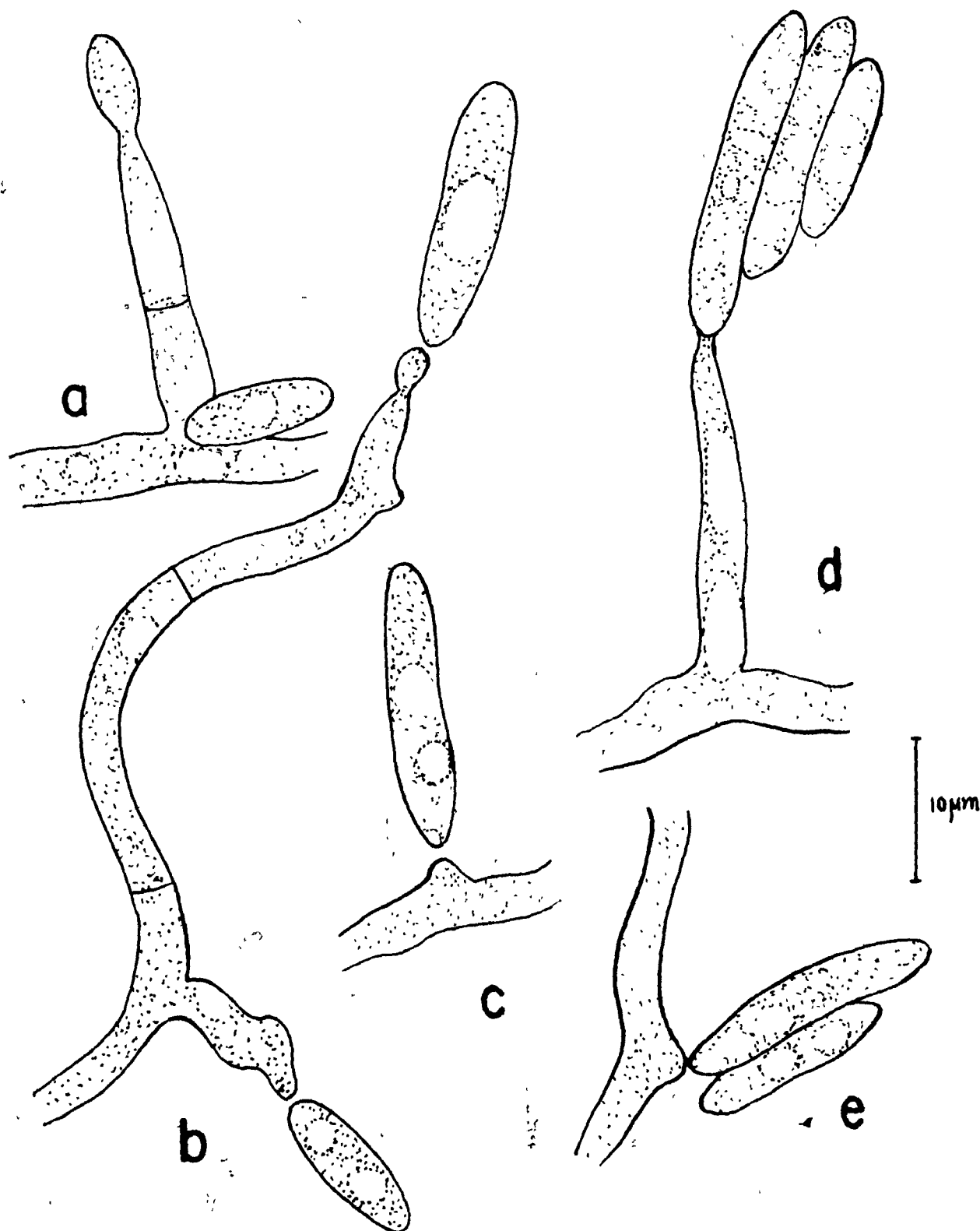


Plate 4. Camera lucida drawings illustrating the types of conidiophores of the stilboid fungus. (a) Septate conidiophore; (b) final branch of hypha serving as a conidiophore; (c) and (e) knob-like conidiophores; and (d) non-septate conidiophore.

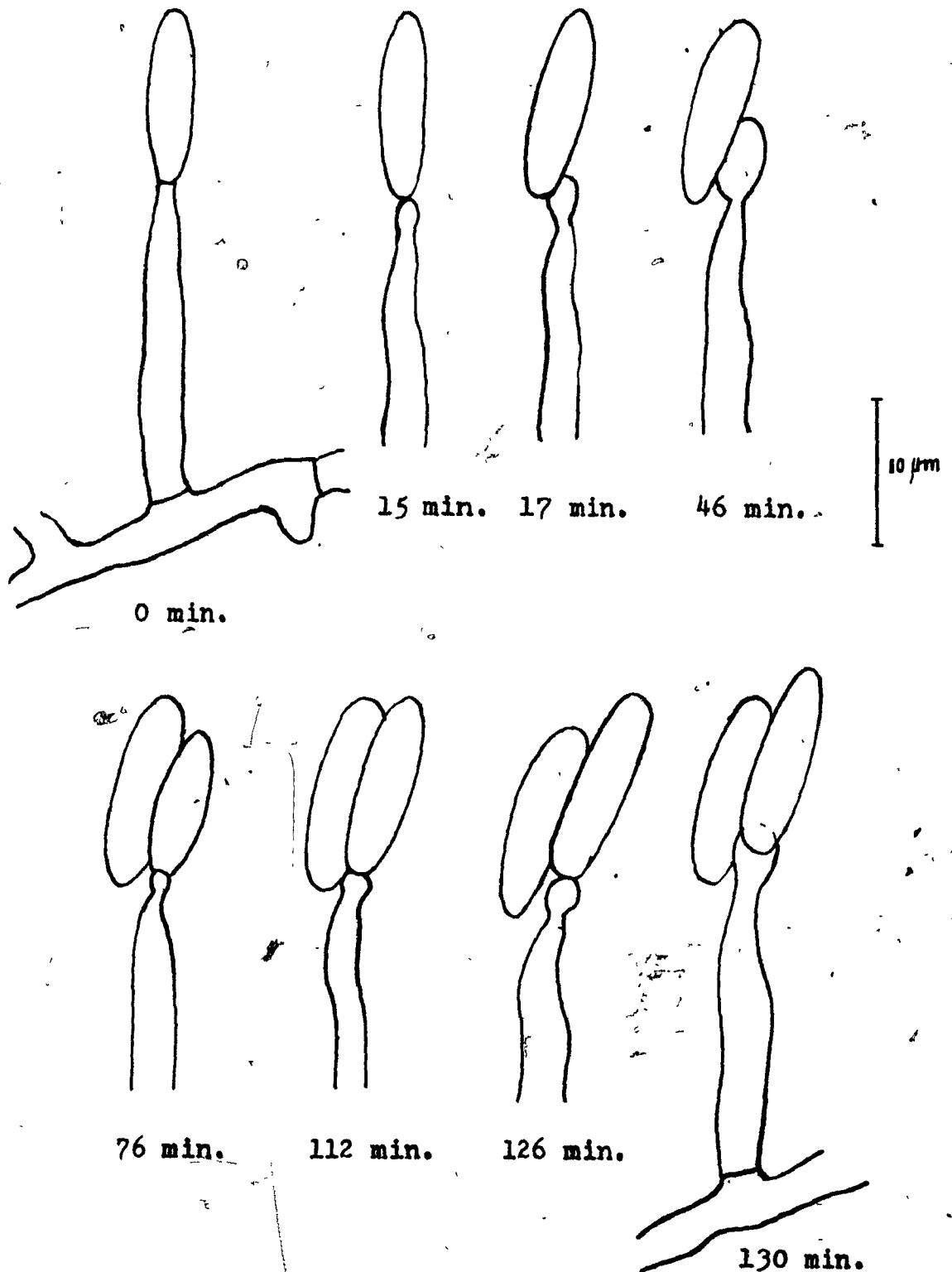


Plate 5. Camera lucida drawings illustrating the stages of conidial formation by the stilboid fungus.

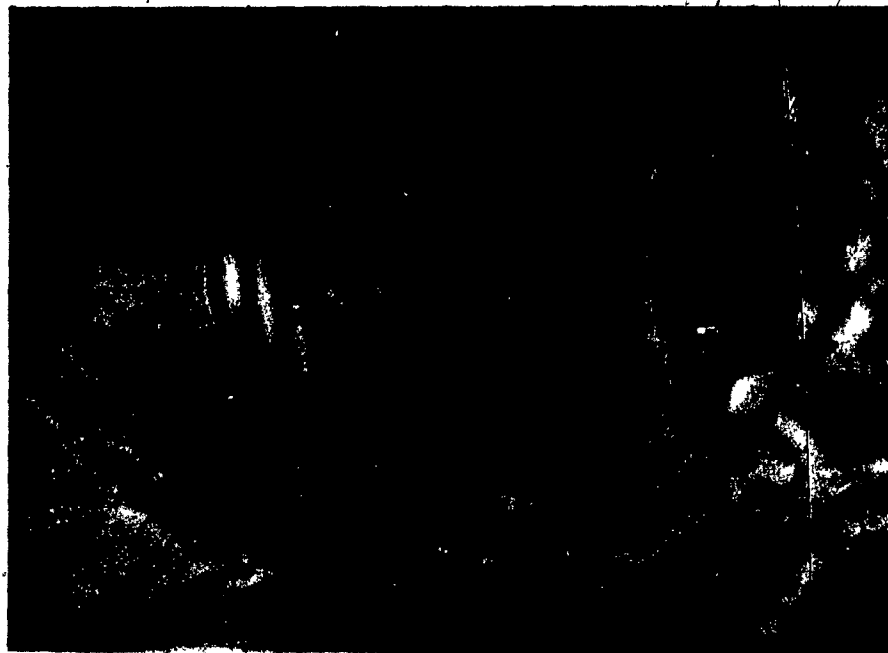


Plate 6. Conidial formation by the stilboid fungus. Mag. 3000X.

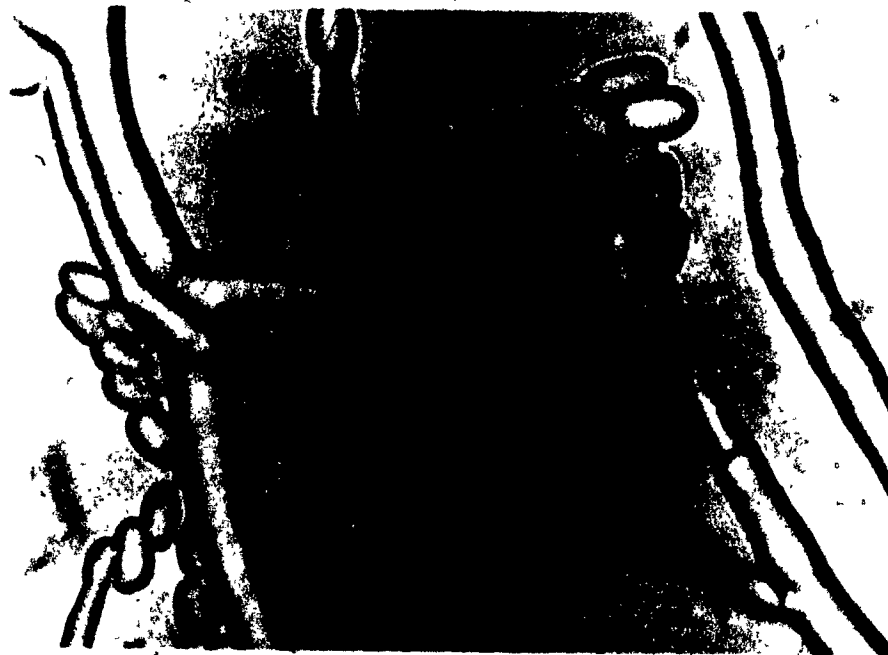


Plate 7. Successive conidia forming a slime-ball. Mag. 3000X.

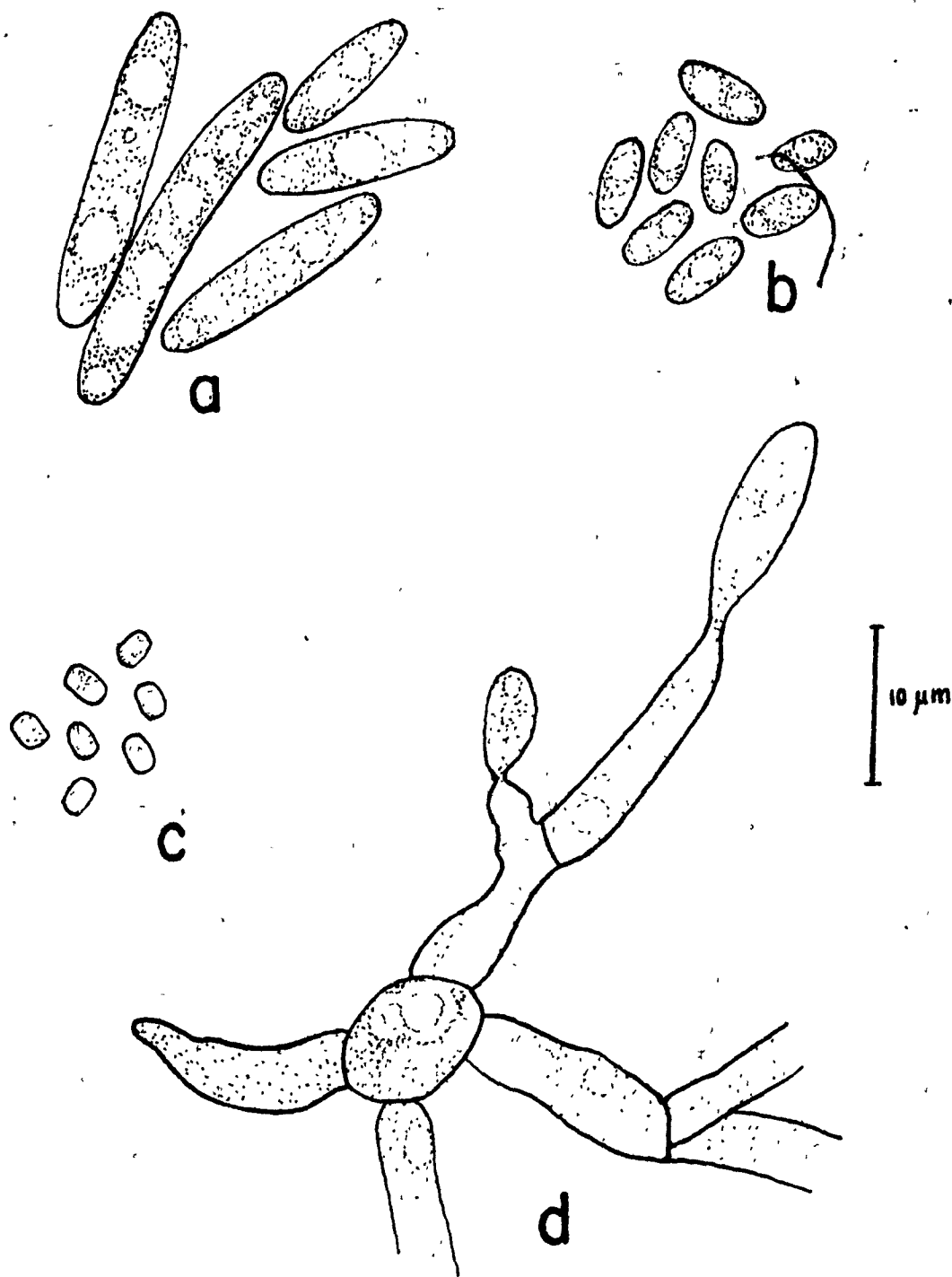


Plate 8. Camera lucida drawings of : (a) - Macroconidia of the stilboid fungus; (b) - microconidia of the stilboid fungus; (c) - conidia of Stilbum zacalloxanthum Moore; and (d) - conidiophore of the stilboid fungus arising directly from a germ tube.

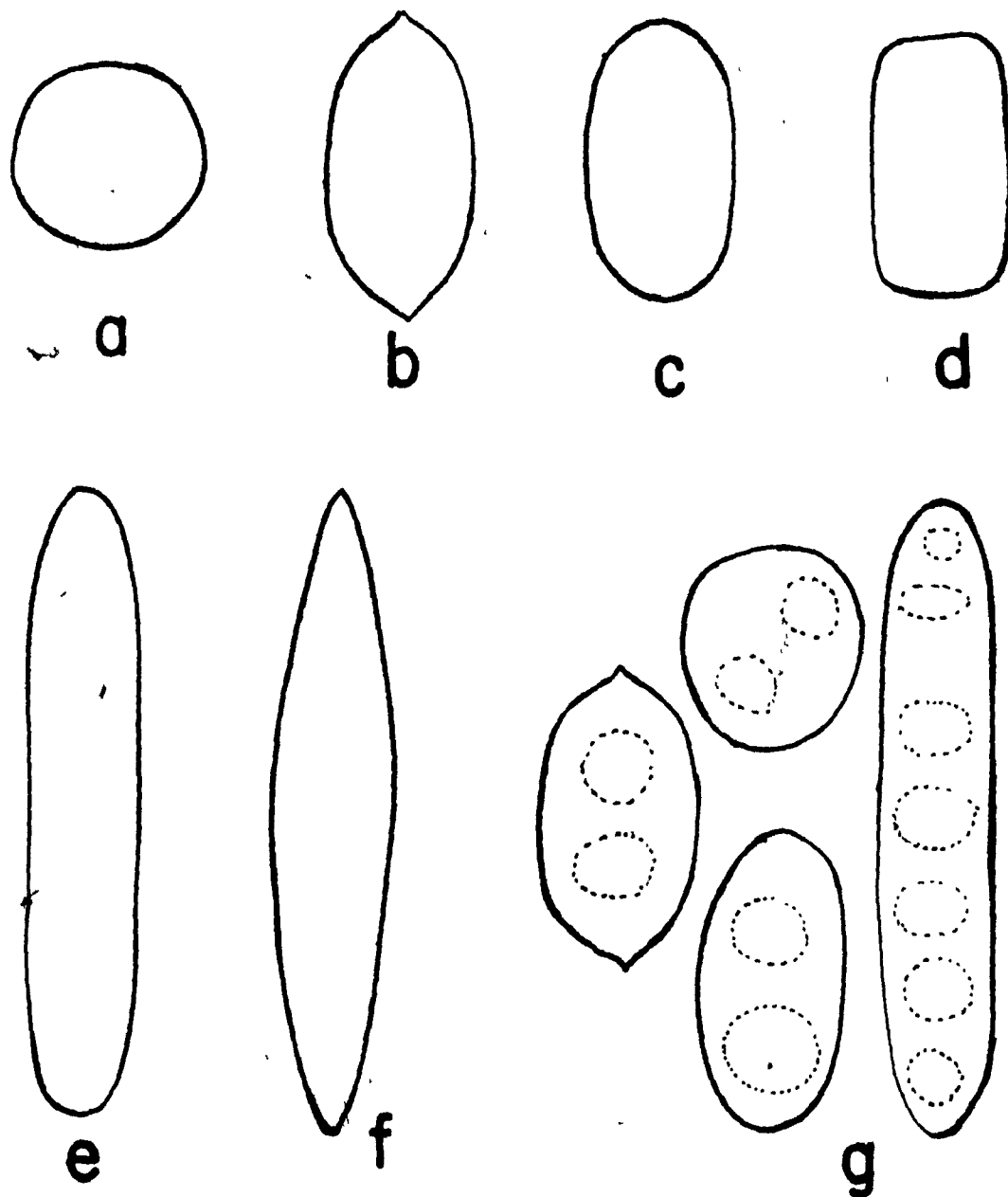


Plate 9. Shape and internal features of conidia: (a) globoid; (b) ellipsoid; (c) oval; (d) oblong; (e) cylindrical; (f) fusoid; (g) guttulations in conidia.

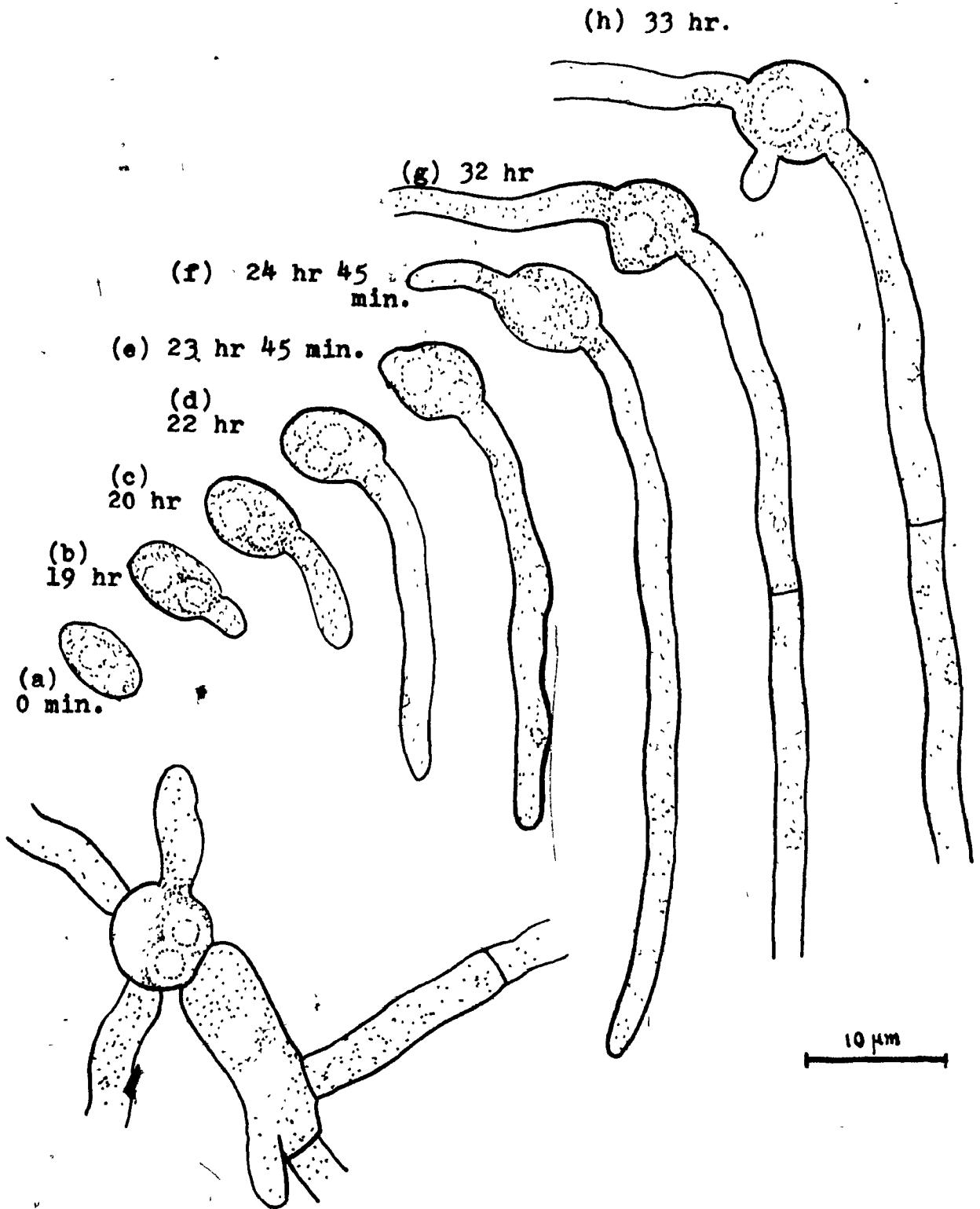


Plate 10. Camera lucida drawings illustrating conidial germination of the stilboid fungus.

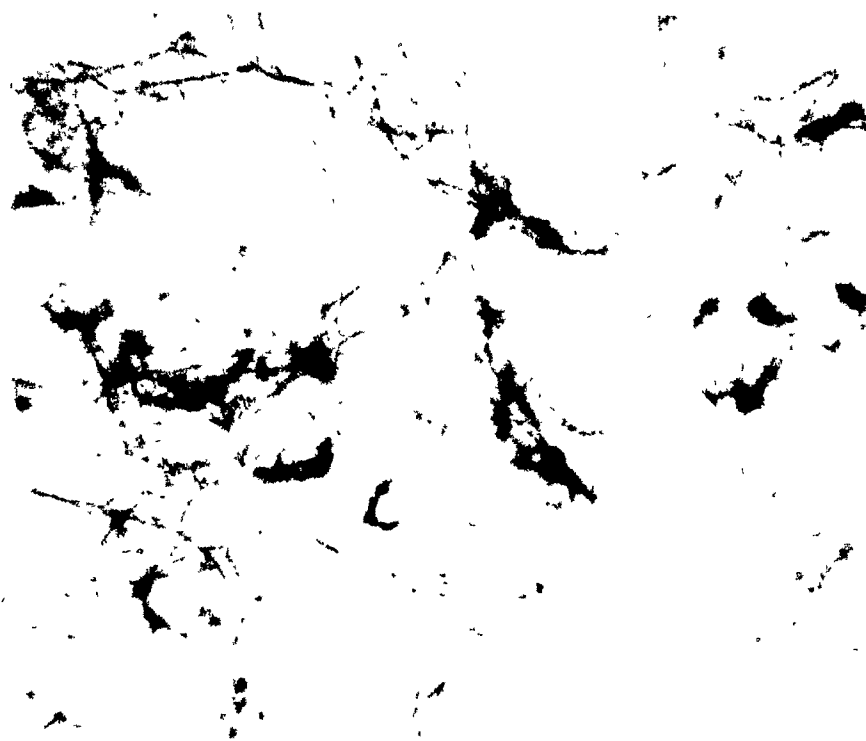


Plate 11. Early stage of hyphae aggregations to form synnemata.
Mag. 250X.



Plate 12. The head of a synnema teased apart to show the production of conidia at the apices of the component hyphae. Mag. 2500X.

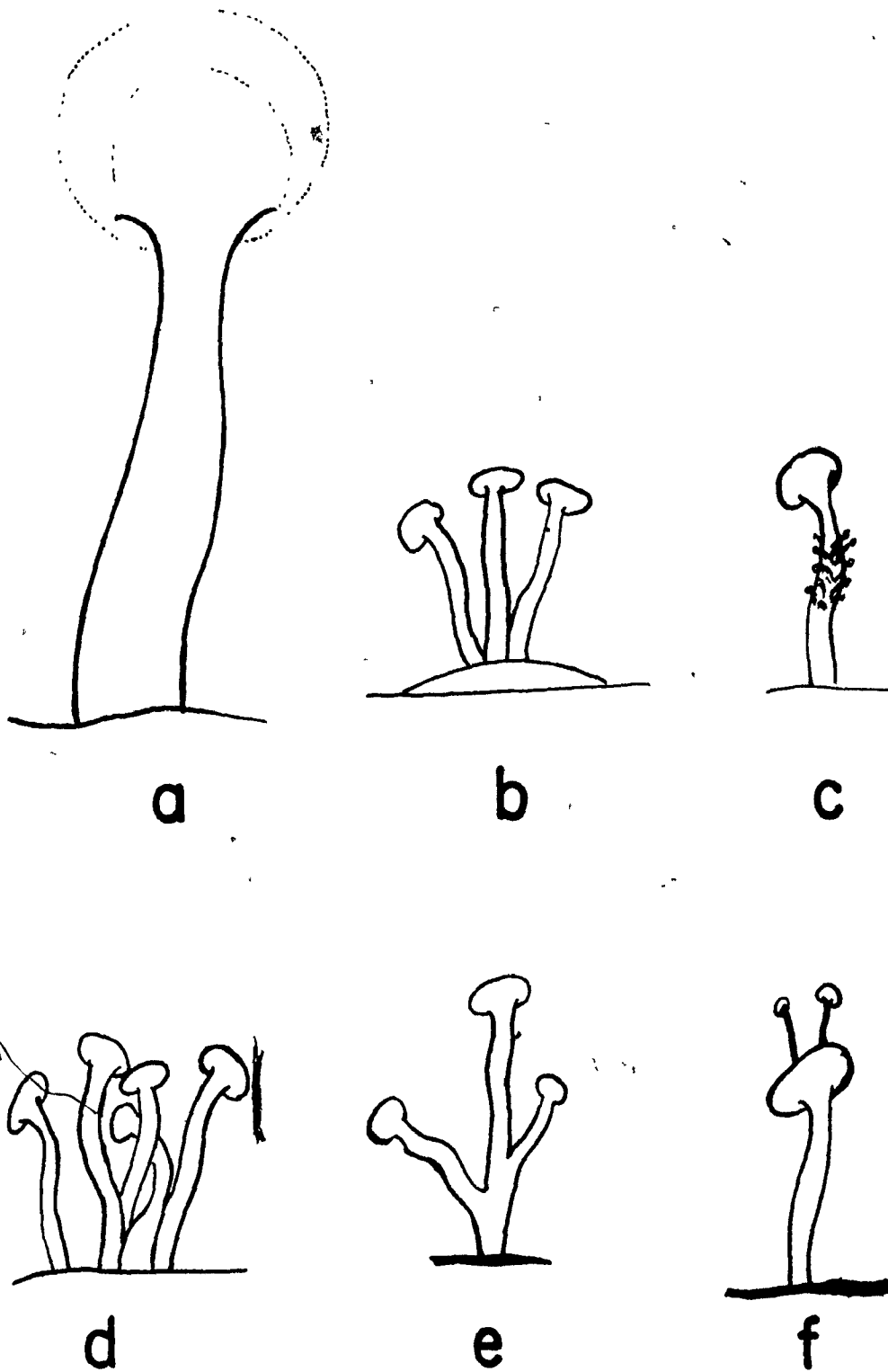


Plate 13. Diagram showing features of synnemata:
 (a) generalized structure of a simple synnema;
 (b) synnemata arising from a stroma;
 (c) hyphae of synnematal stalk producing conidia;
 (d) synnema produced in a group
 (e) synnema with lateral branches; and
 (f) secondary synnemata on the head of a primary synnema.



* Plate 14. Primary synnema growing from inoculated soil, producing a small secondary synnema from its head. Mag. 300X.

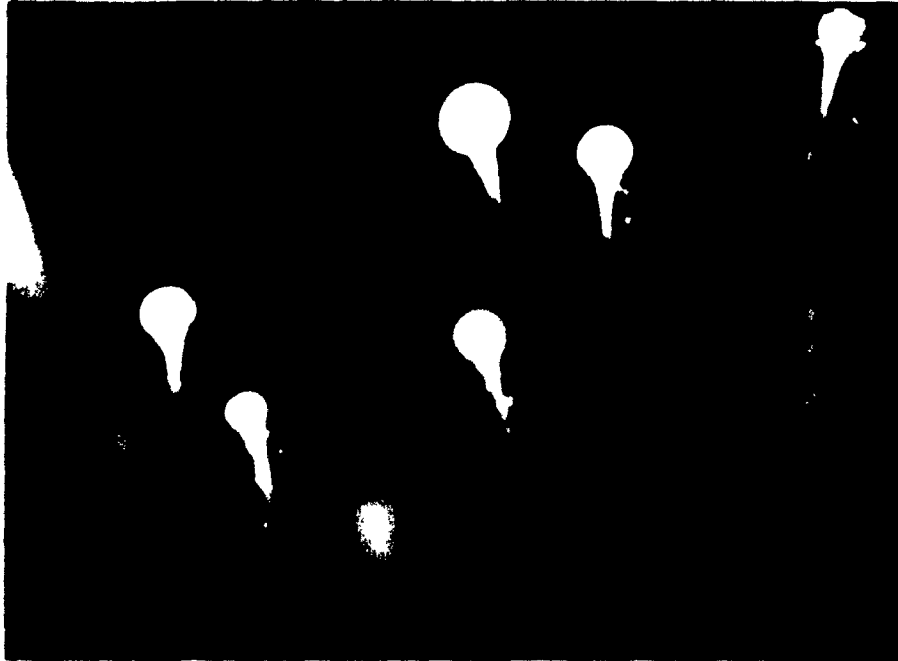


Plate 15. Some hyphae of the synnematal stalks producing conidia.



Plate 16. Synnemata of the stilboid fungus growing from clover. Mag. 1500X.



Plate 17. Synnemata of the stilboid fungus arising from stroma on potato dextrose agar. Mag. 1600X.

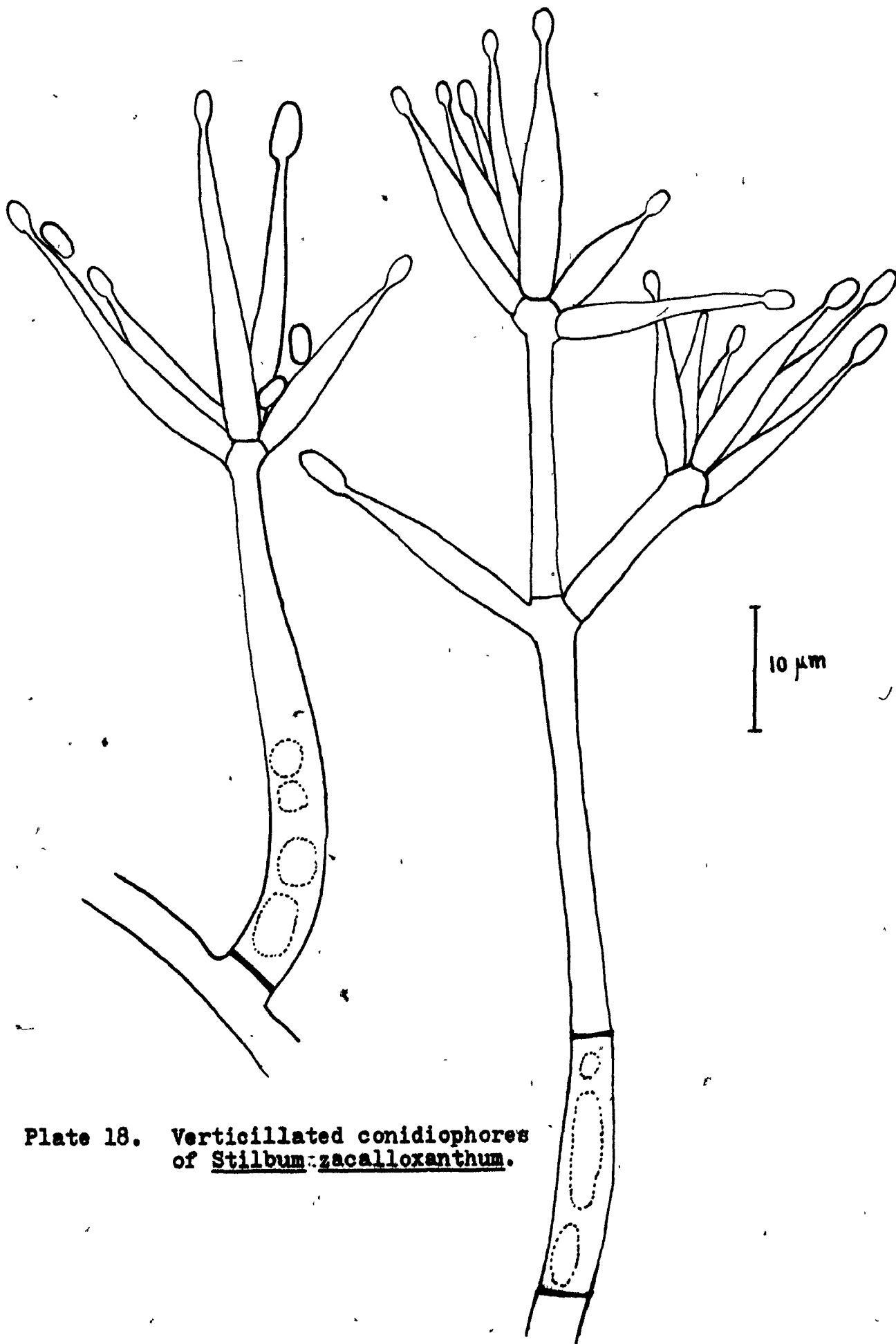


Plate 18. Verticillated conidiophores
of *Stilbum zacalloxanthum*.

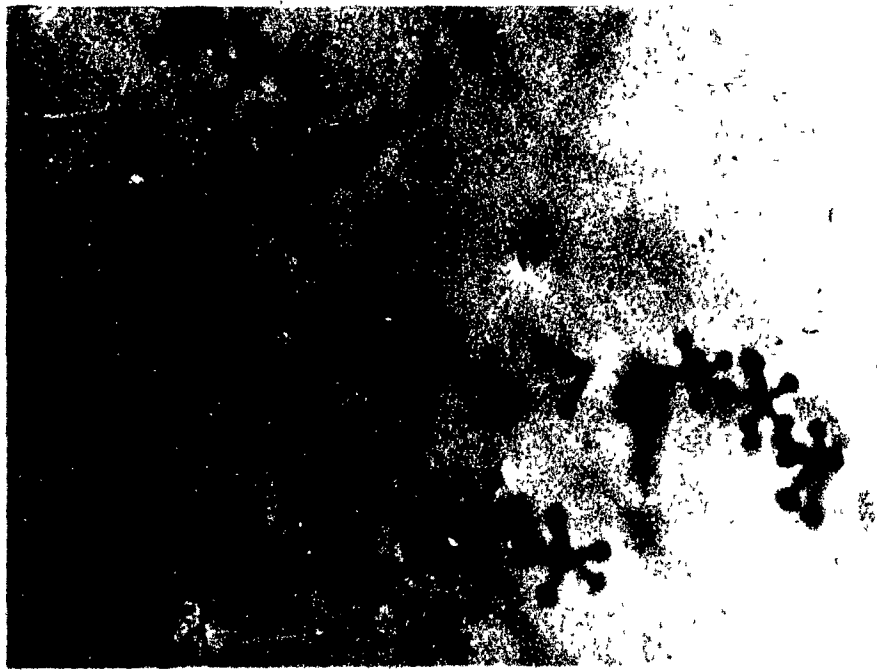


Plate 19. Mag. 300X.

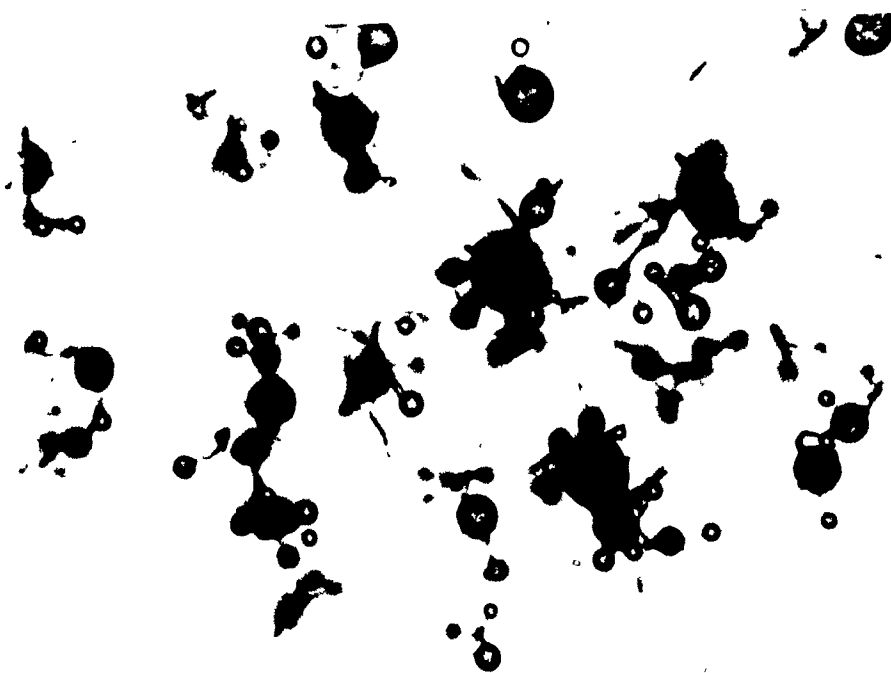


Plate 20. Mag. 1800X

Plate 19 and 20. Clusters of conidia produced on verticillate conidiophores of Stilbum zacalloxanthum Moore.

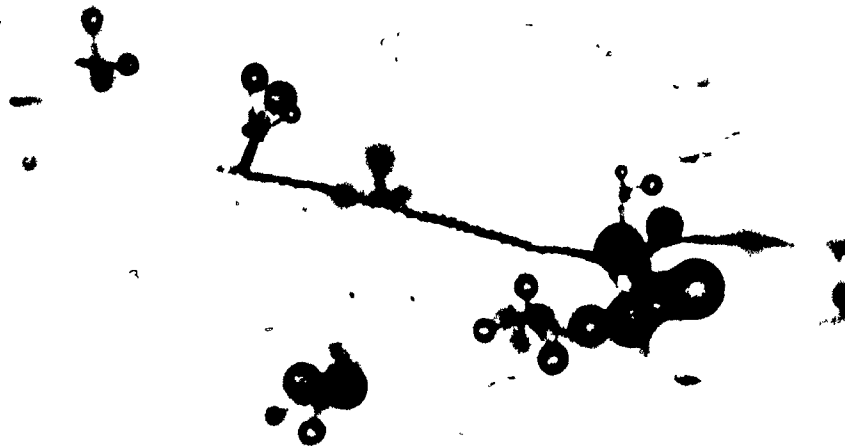


Plate 21. Tiny droplets adhering to conidiophores and aerial hyphae of Stilbum zacalloxanthum Moore. Mag. 1800X.

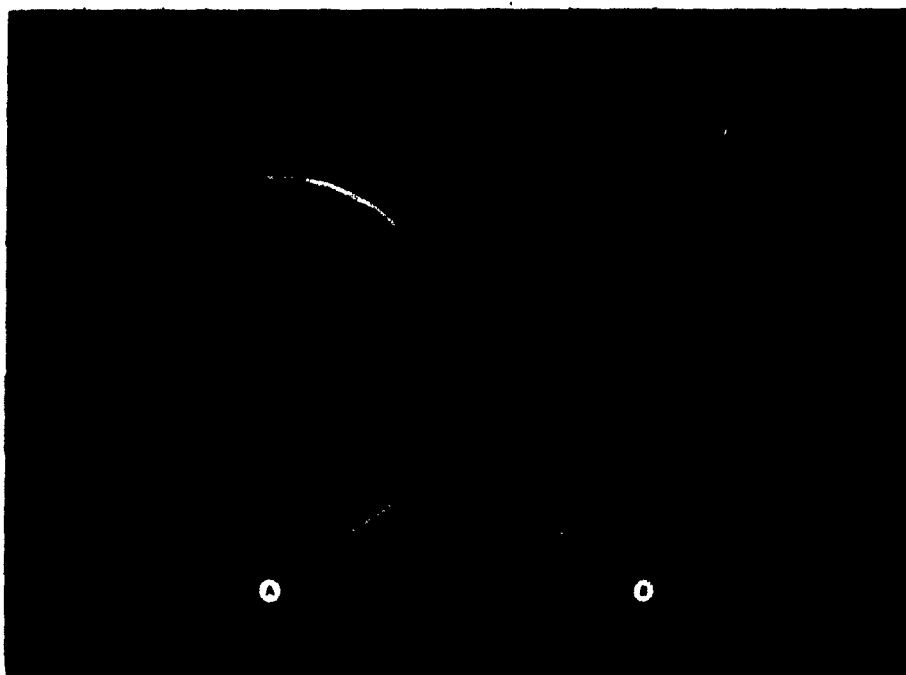


Plate 22. Agar cultures of (A) the stilboid fungus, and
(B) Stilbum racalloxanthum Moore,

Table 1. The effect of temperature on growth and sporulation of the stilbeid fungus on potato dextrose agar for 18 days in continuous darkness.

Temperature (°C)	Mycelial growth*	Spores per colony x 10 ⁶
	mm	
10	16	18
15	23	120
20	55	269
25	62	376
28	61	328
32	39	58
40	0	0

* Average diameter of five colonies.

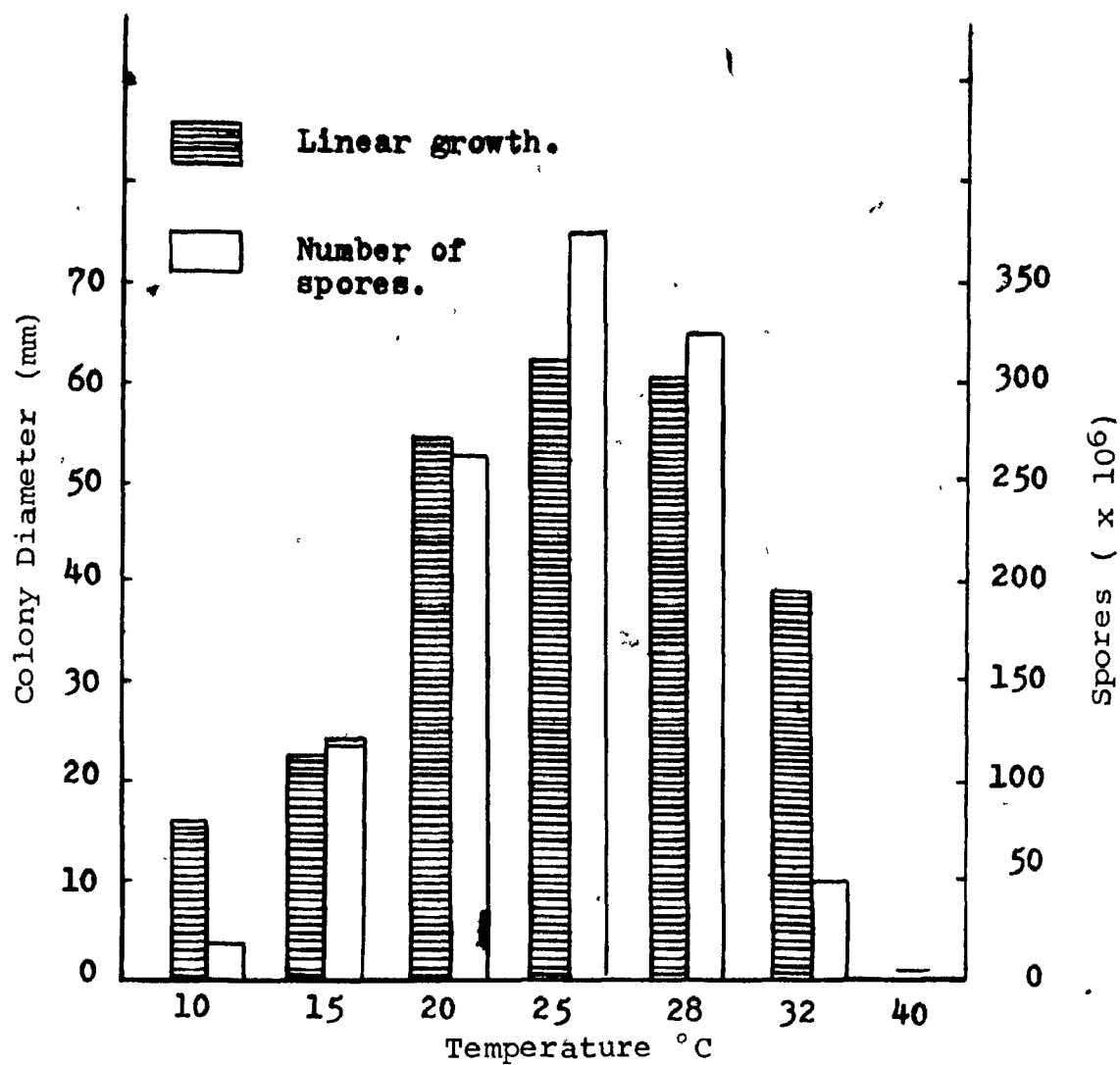


Figure 1. Growth and sporulation of the stilbeid fungus on potato dextrose agar for 18 days in continuous darkness at different temperatures.

B. FACTORS AFFECTING GROWTH AND SPORULATION OF THE STILBOID FUNGI

1. Temperature

Table 1 and Figure 1 show that of the test temperatures between 10 and 40 degrees centigrade, the optimum for growth and sporulation of the stilboid fungus in continuous darkness was 25°. The fungus grew well between 25 and 28 degrees centigrade. No growth occurred at 40° and there was little growth at 10°.

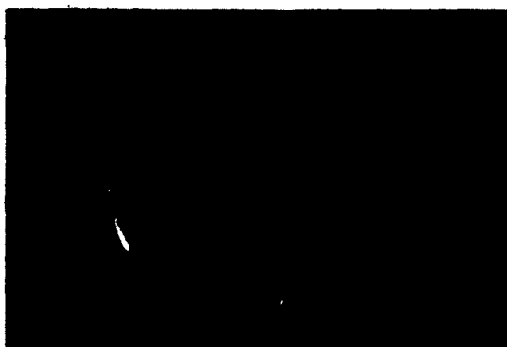
Plate 23 and 24 show the appearance of the cultures grown at 10 to 32 degrees centigrade. The cultures grown at 15° and 20° appeared white in colour with only a few whitish synnemata. More spores, on more evenly spaced synnemata, were produced at 20° than at 15°. The colour of the cultures kept at 25° was dark cream to very light purple, and they produced a lot of aerial hyphae and synnemata while those at 10, 28, and 32 degrees centigrade were relatively dark purple and had less aerial hyphae and fewer synnemata.

Plate 25 shows the appearance of the stilboid fungus grown on PDA in continuous darkness, but with the temperature alternating between 20° and 25° at 12 hour intervals. The growth and sporulation of the fungus in this environment averaged 6.0 mm in diameter with 130×10^6 conidia per colony in 18 days. There was only slight evidence of zonation of aggregated hyphae with only a few whitish synnemata in alternate zones.

In summary, the optimum temperature for the growth and sporulation of the unnamed stilboid fungus in continuous darkness was 25°. Zonation of the culture did not occur when it was grown at any constant temperature level from 10 to 32 degrees centigrade. Alternating the temperatures of 20 and 25 degrees centigrade at 12 hour intervals produced some evidence of a corresponding growth rhythm.



a.



b.



c.

Plate 23. Cultures of the stilboid fungus grown in continuous darkness at (a) 15° ; (b) 20° ; and (c) 28° , on potato dextrose agar for 18 days.

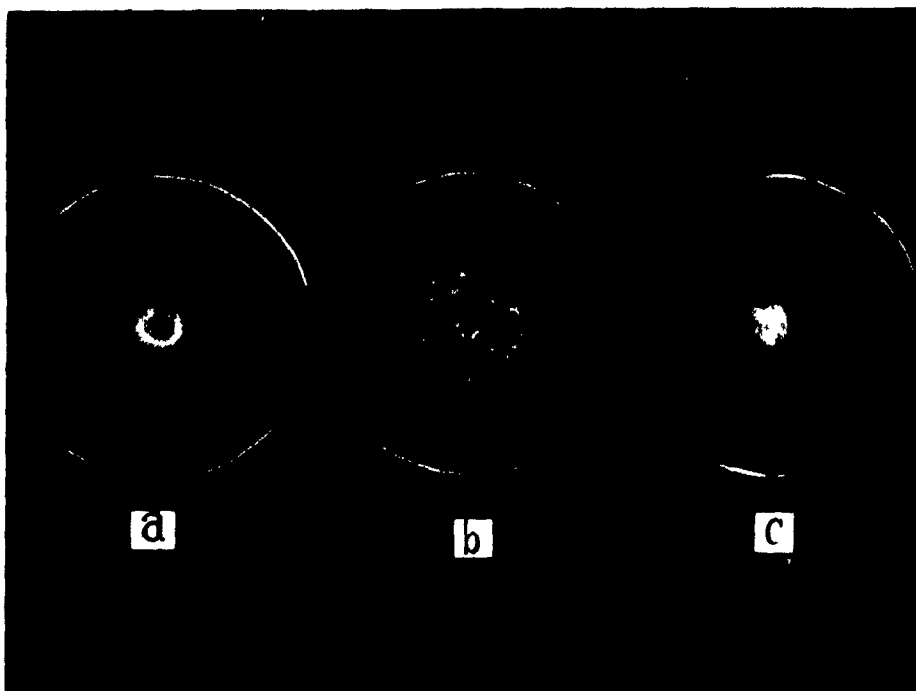


Plate 24. Cultures of the stilboid fungus grown at (a) 10° ; (b) 25° ; and (c) 32° , on potato dextrose agar for 18 days in continuous darkness.



Plate 25. A culture of the stilboid fungus grown in continuous darkness on potato dextrose agar with alternating temperatures of 20° and 25° at 12 hour intervals for 18 days.

Table 2. Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in continuous darkness and alternating periods of light and dark for 17 days at 25 degree centigrade.

Nutrient media*	pH of media	Cont. darkness		Alt. light-dark***	
		Mycelial growth**	Spore per colony x 10 ⁶	Mycelial growth**	Spore per colony x 10 ⁶
		mm		mm	
CMA	5.6	67	118	75	38
CSA	7.3	60	2,340	62	5,300
LBA	5.6	70	152	77	90
MA	7.0	56	1,703	65	2,175
MEA	4.6	52	653	55	660
NA	6.8	50	268	51	210
PDA	5.6	56	565	57	580

* CMA-cornmeal agar; CSA-czapek solution agar; LBA-lima bean agar; MA-mycological agar; MEA-malt extract agar; NA-nutrient agar; PDA-potato dextrose agar;

** Diameter of the colonies in millimeters.

*** 12-hr light - 12-hr dark intervals with light intensity of 15,000 ft-candles.

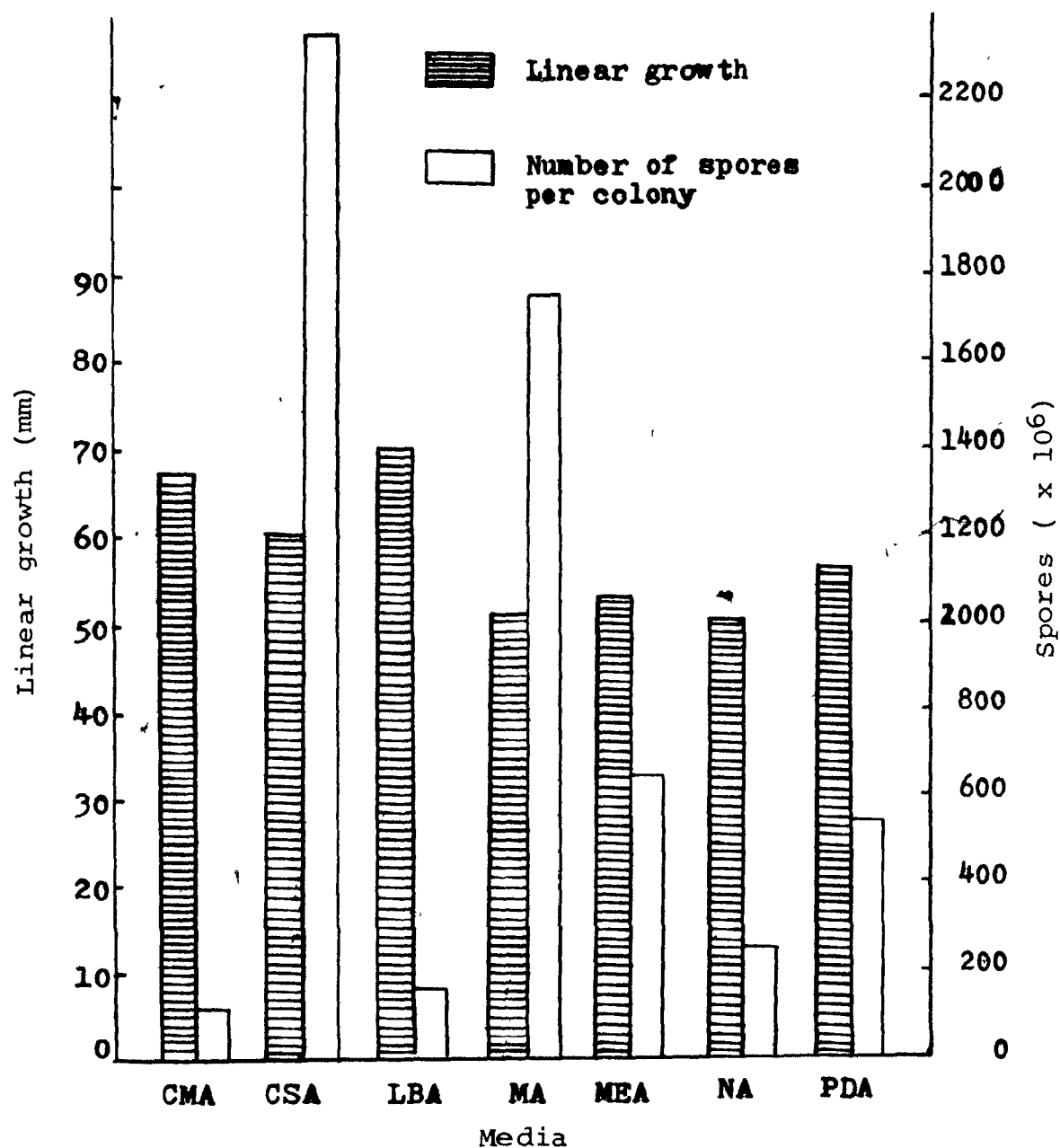


Figure 2. Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in continuous darkness for 17 days at 25°.

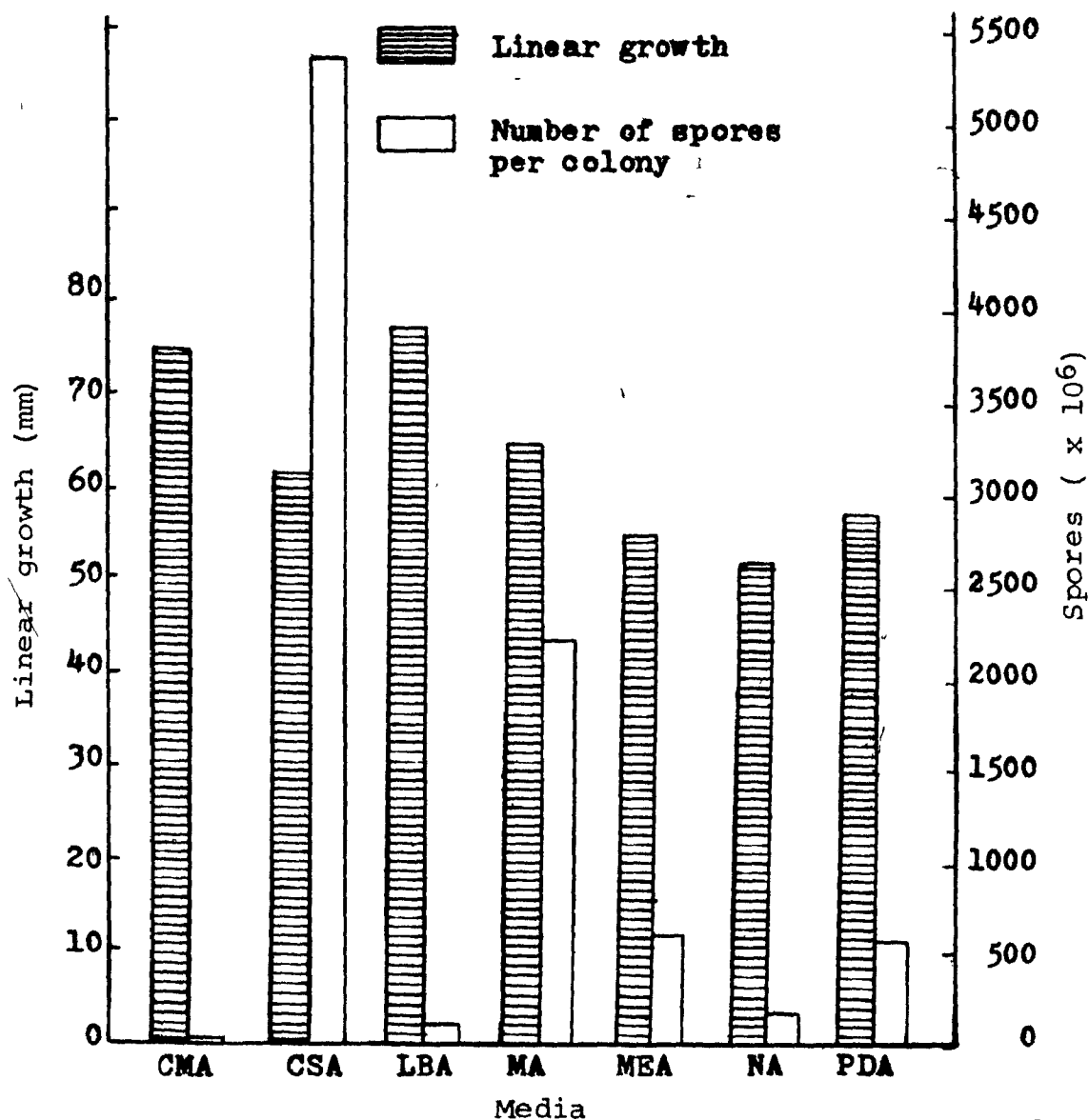


Figure 3. Growth and sporulation of the stilboid fungus on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°.

Table 3. Growth and sporulation of Stilbum zacalloxanthum Moore on seven kinds of nutrient media in continuous darkness and alternating periods of light and dark for 17 days at 25 degree centigrade.

Nutrient media*	pH of media	Cont. darkness		Alt. light-dark***	
		Mycelial growth**	Spore per colony $\times 10^6$	Mycelial growth**	Spore per colony $\times 10^6$
		mm		mm	
CMA	5.6	90	112	74	40
CSA	7.3	85	59	45	39
LBA	5.6	87	316	65	221
MA	7.0	81	3,450	55	3,488
MEA	7.0	32	345	28	394
NA	6.8	37	93	32	235
PDA	5.6	62	1,279	62	645

* CMA-corn meal agar; CSA-czapek solution agar; LBA-lima bean agar; MA-mycological agar; MEA-malt extract agar; NA-nutrient agar; PDA-potato dextrose agar.

** Diameter of colonies in millimeters.

*** 12-hr light - 12-hr dark intervals with light intensity of 15,000 ft-candles.

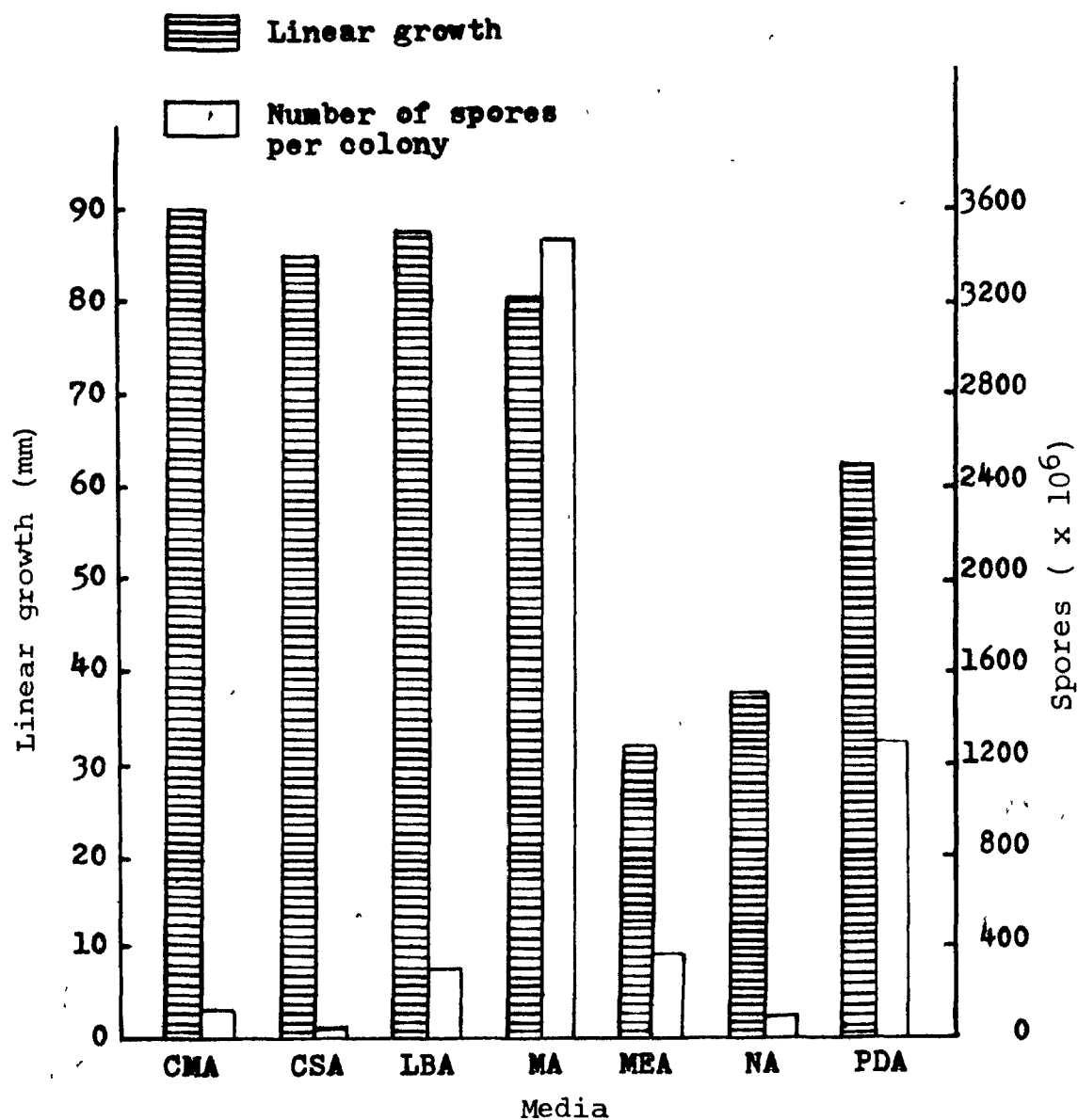


Figure 4. Growth and sporulation of *Stilbum zacallexanthum* on seven kinds of nutrient media for 17 days in continuous darkness at 25°.

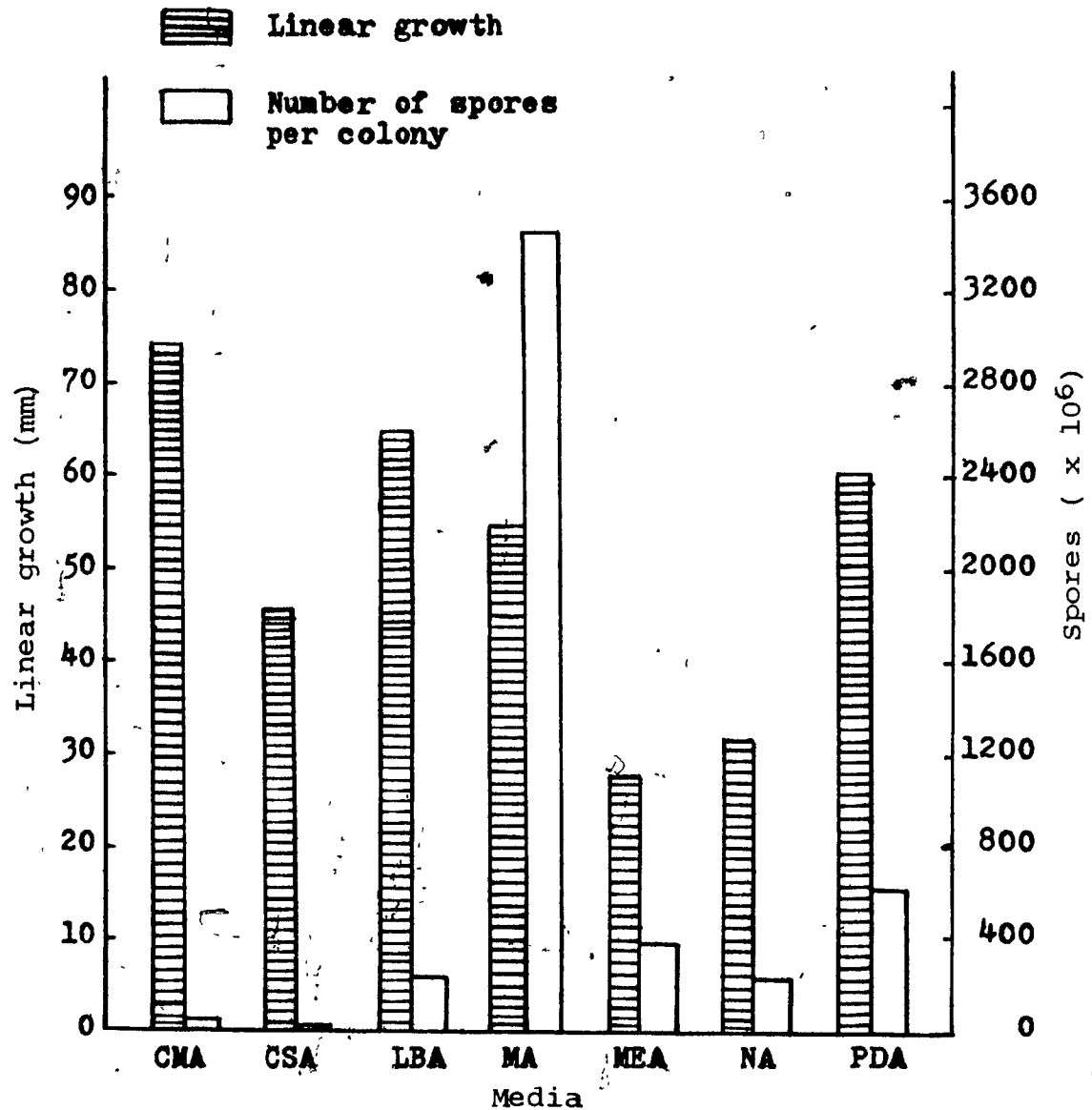


Figure 5. Growth and sperulation of *Stilbum sacalloxanthum* on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°.

2. Media

The purpose of this study was to find an appropriate medium and to learn more about the nutritional requirements of the stilboid fungus and to see if they were different from those for Stilbum zacalloxanthum. It was also to determine the effects of certain nutritional factors on growth and sporulation of the two fungi. The following results show that the stilboid fungus and S. zacalloxanthum differed greatly in their growth rates, sporulation and colony appearance on the different nutrient media tested:

a. Growth and Sporulation

i. The stilboid fungus

From Table 2, Figure 2 and Figure 3, it may be seen that the maximum number of spores was produced on czapek solution agar (CSA) whereas the maximum linear growth usually occurred on lima bean agar (LBA) in both continuous darkness and alternating light-dark periods. In both conditions the number of spores produced decreased from czapek solution agar (CSA), mycological agar (MA), malt extract agar (MEA), potato dextrose agar (PDA), nutrient agar (NA), lima bean agar (LBA), to corn meal agar (CMA) respectively. Spore numbers were higher on colonies in alternating light-dark periods than on those in continuous darkness for CSA, MA, MEA, and PDA, while those on CMA, LBA, and NA had fewer spores in alternating light-dark periods than those in continuous darkness.

Colony diameters were greatest on LBA and decreased respectively from CMA, CSA, MA, PDA, MEA, to NA. On PDA and MA, the mycelial growth was equal but sporulation was about twice as great on MA as on PDA. The cultures on CMA and LBA had colonies of the largest diameter while spore production was at a minimum. The linear growth of cultures in alternating light-dark periods was always greater than those in continuous darkness. This may be because these media have little, if any, sugar, containing only the infusion of corn meal and lima bean respectively. The fungus seems to have responded better to the saccharose contained in CSA, than to the dextrose contained in PDA and MA as it grew faster and produced more spores on the former than on the latter, although factors other than the sugar may have been responsible for the observed differences in growth and sporulation.

ii. Stilbum zacalloxanthum Moore

Maximum spore production occurred on MA whereas the maximum mycelial growth occurred on CMA in both continuous darkness and alternating light-dark conditions. The data of Table 3 and Figure 3 show that sporulation decreased respectively from MA, PDA, MEA, LBA, CMA, NA to CSA when the fungus grew in continuous darkness, and from MA, PDA, MEA, NA, LBA, CMA, to CSA when it was in alternating light-dark periods. Sporulation was poor on NA, CMA, and specially on CSA. Unlike the unnamed stilboid fungus, spore production

of S. zacalloxanthum, under continuous darkness, was usually a little higher than it was in alternating light-dark periods on most media except for MEA and NA on which more spores were produced in the later condition.

The diameter of the colonies of S. zacalloxanthum was greatest on CMA, followed by LBA, CSA, MA, PDA, NA, and MEA respectively in both light conditions. However, the colonies on CSA grew slower in alternating light-dark periods. It grew at the same rate on PDA in both light conditions. In general S. zacalloxanthum grew faster and sporulated sooner than did the unnamed stilboid fungus under similar environmental conditions.

b. The characteristics of the cultures

i. The unnamed stilboid fungus

The appearance of the colonies of the stilboid fungus on seven kinds of nutrient media at 25° in continuous darkness and alternating light-dark periods for 17 days are shown in Plates 26 and 27.

In continuous darkness the fungus produced a superficial purplish mycelium on PDA with abundant synnemata and spores at the center of the culture and sporodochia at the periphery. The colour of the mycelium gave a purplish hue to the whole culture. On CSA, the colony was light yellow and produced about five zonations in 18 days (Plate 28). The culture was compact and possessed abundant yellowish synnemata

The heads of the synnemata were small, relatively dry and joined together within the same ring of zonation. The colony grown on NA was creamy in colour with only one zone of synnemata and spores around the inoculated area. Sporodochia or stromata were scattered thinly throughout the culture. The colonies grown on CMA and LBA were more or less colourless except for several whitish synnemata. The synnemata had stalks up to 5 mm long when produced on CMA and sometimes they fell flat on the medium. The culture on MA was bright yellow with a large number of synnemata occurring in alternate zones. The heads of these synnemata were larger (averaging about 2.5 mm in diameter) than any produced on other media. They were so slimy that they looked like drops of fluid and they were all joined together in each zonation. The appearance of the colony grown on MEA was different from the others. Only two zones or rings were produced, each consisting of long-stalked synnemata. The synnemata produced in the central part of the colony were whitish in colour. There were radial lines of mycelia in the cultures, some of which had more synnemata than others.

When grown at constant temperature, in alternating periods of light and darkness zonation occurred in all cultures, on all media. (Plate 27). Most of the cultures produced about 14 zonations in 17 days. Cultures on CSA, MA and PDA were bright yellow while those on MEA, LBA, CMA, and NA were almost colourless except for the yellowish synnemata. Zonation was

very evident on PDA, MA, and CSA. In the case of CSA, zonation was more compact. There were eight distinct zones, about 1 mm apart (except for the first two which were broader) near the center of the culture. The area of the culture outward from these eight zones was more uneven, with less evidence of zonation and there was some white colour at the periphery (Plate 28). On MA, the synnemata within the same zone were gathered together to make it about 1 mm wide. The clear area between each zonation was also about 1 mm wide. The heads of the synnemata were dry and of a bright yellowish colour. The colonies often showed evidence of mutation. The mutated sections appeared as ray-like patches of a different colour, and usually with fewer and smaller synnemata, but the lines of zonation could still be seen. Cultures grown on PDA were similar to those on MA. Their zonations were made of bright yellowish masses of sporodochia and synnemata (Plate 29), except at the periphery of the colonies where they tended to be white. The synnemata usually developed from the sporodochia. Only the center of the colony grown on NA was yellow while the rest of it was colourless or white. The yellowish colour was made of the fine synnemata forming zonations. Outward from the eighth ring, only indistinct zones were observed. Probably this is because spores had not yet been produced in the newer mycelium. The cultures on MEA had only five zonations at the center of the colonies made of dry-headed yellowish, synnemata. In the area outward

from the fifth ring, the colony was sectored and the zones were uneven and less distinct. The cultures of the fungus grown on LBA and CMA were similar in colour. They were white with light yellowish dried synnemata heads. Zonation was more evident on LBA than on the CMA where very few synnemata occurred.

ii. Stilbum zacalloxanthum

In continuous darkness (Plate 30), the cultures of S. zacalloxanthum on PDA were of a lighter yellow than those grown in alternating light-dark periods. Whitish mucilaginous droplets of spores were abundant, some having diameters up to 2 mm, on bright yellowish stalks. Most of these big mucoid heads, looking like droplets of fluid, were at the center of the cultures. The newly formed synnemata at the periphery were often without heads. Most of the synnemata produced on CSA had small mucoid heads of about 29 micrometers in diameter, but there were a few large ones. The size of the synnematal stalks varied with the size of the heads, those with small heads having correspondingly small stalks. The cultures had a purplish colour, and lacked zonation when grown in continuous darkness. In this respect S. zacalloxanthum was similar to the unnamed stilboid fungus. The culture on NA was smaller than on other media, and it was brownish in colour containing abundant fine whitish masses of conidia. Whitish synnemata were produced only at the center of the

culture where inoculation took place. This was surrounded by a clear zone containing only mycelia without spore bearing structures. In the area outside of this clear circle, there were many small mucoid heads of conidia produced on branched conidiophores. Their microscopic appearance shown in Plate 19 and 20 (page 53). S. zacalloxanthum was similar on CMA and LBA, the only difference being that the stalks of the synnemata produced on CMA were yellow while those on LBA were white. Both cultures had whitish slimy droplets of spores on the heads of synnemata, which were more abundant on CMA than on LBA. No zonation occurred in either medium. The cultures grown on MA appeared the same as those on PDA except for the colour, which was red to purple on MA and bright yellow on PDA. The synnemata on MA produced white mucoid heads, on reddish stalks, as big as those on PDA. All of the large droplets were produced at the center and the smaller ones were at the periphery. The yellowish colour appeared only at the center of the culture grown on MEA, where the mucoid heads were about the same size as those on MA. Only two distinct zonations occurred, and these were surrounded by a whitish band in which a few young yellowish stalks were forming. This nearly colourless band around the zoned part of the colony was about 2 mm wide. This synnemata-less peripheral zone sometimes occurred on PDA and MA also. However, it was only on MEA that S. zacalloxanthum produced a distinctive zonation when grown in continuous darkness.

In alternating light-dark periods, zonation occurred in all media tested (Plate 31). The cultures grown on PDA appeared bright yellow because of the large number of bright yellowish synnemata, even though their heads consisted of whitish slimy droplets of spores. Three or four rows of the slimy heads of spores commonly ran together to produce one continuous wide zone, separated from a similar zone of aggregated heads of synnemata by a non-sporulating band of vegetative mycelium. This is shown in Plate 32. The margins of the colonies were usually uneven. Fourteen rings or zones were produced in the 17-day test period, the first one being produced on the fourth day. The number of zonations was the same in all media. The characteristics of the cultures grown on MEA were similar to those on PDA, except that the clear zone at the margin of the colony was more pronounced. The general colony appearance of the cultures grown on CMA was similar to those on LBA. They grew faster on both of these than on any other media. The cultures appeared colourless with whitish stalks and heads of synnemata. The heads of the synnemata, each of which was independent of the others, consisted of shiny droplets of conidia. The synnemata developed close together in zones, which were about 3 to 4 millimeters from one another. On NA, the cultures were white with whitish synnemata. Each zone of short-stalked synnemata was about 1 mm from the succeeding zone. The cultures on MA were reddish purple in colour. The stalks of the synnemata were also

purple with large heads of light pinkish droplets of conidia. These droplets looked like fluid and they were easily dispersed when shaken. They were commonly joined together so that one large drop covered many stalks. Zones of synnemata 3 mm wide formed in response to the light periods of the diurnal cycles. The cultures on CSA were of a light brownish colour, on which only a few whitish synnemata were produced. Consequently, zonation was not readily evident although 14 narrow rings could be seen in some cultures on this medium.

Under the experimental conditions of this test, CSA and MA were the most appropriate media for growth and sporulation of both the unnamed stilboid fungus and S. zacalloxanthum. The results show that these two fungi do, however, respond differently from one another on various media. Data on their comparative growth and sporulation are shown in figures 6 to 9.

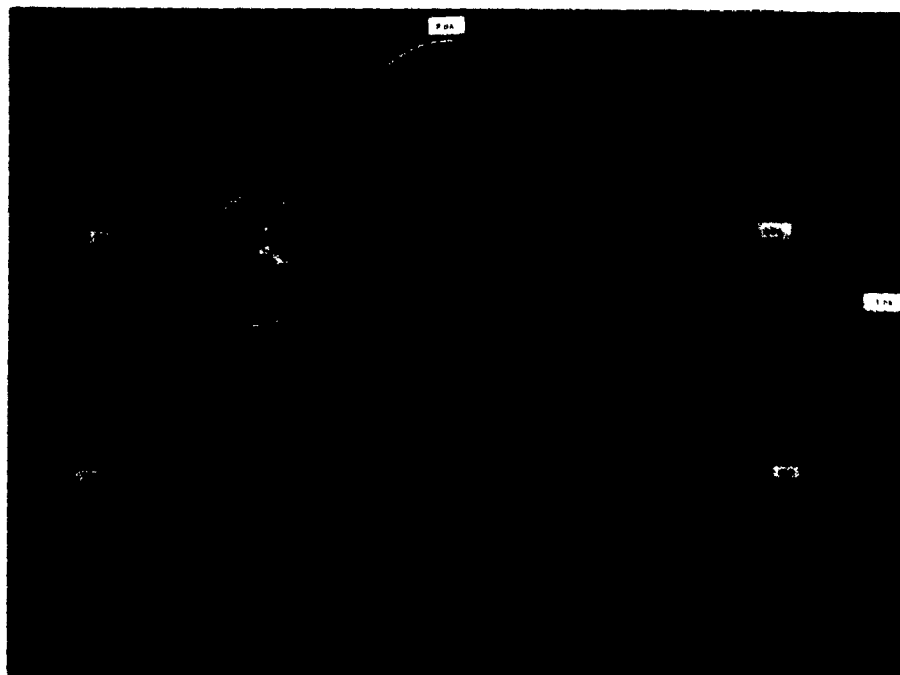


Plate 26. The growth and colony appearance of the stilboid fungus on 7 kinds of media in continuous darkness at 25° for 17 days.

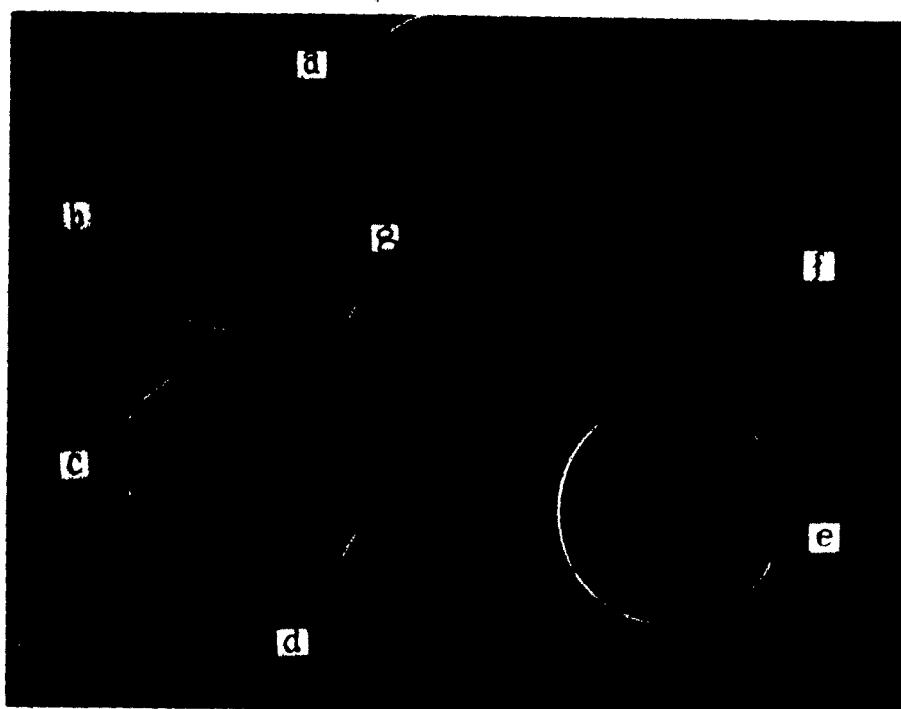


Plate 27. The growth and colony appearance of the stilboid fungus on 7 kinds of media in alternating light-dark periods at 25° for 17 days. (a-PDA; b-CSA; c-LBA; d-MA; e-CMA; f-NA; and g-MEA).

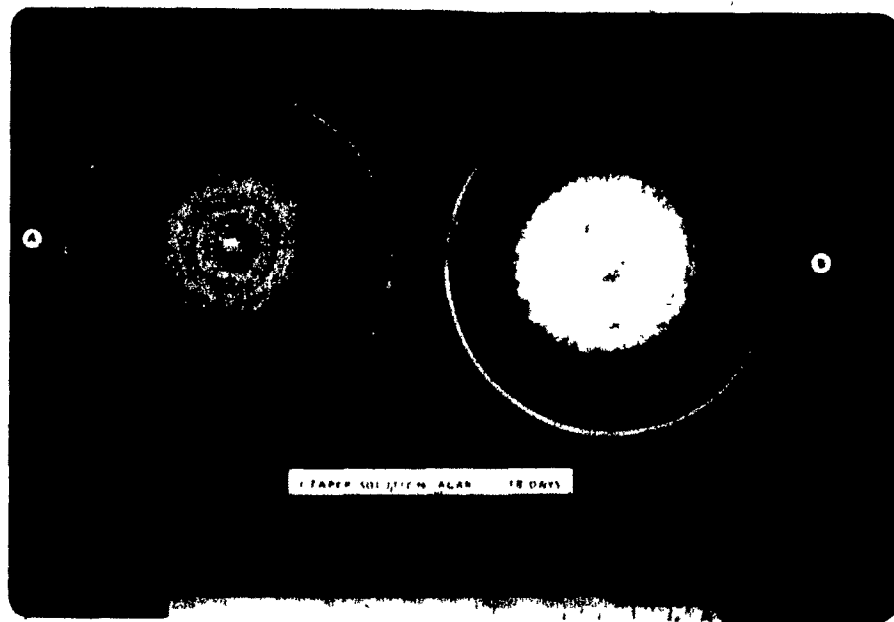


Plate 28. The cultures of the stilboid fungus on Czapek solution agar; (A) in alternating light-dark and (B) in continuous darkness, for 18 days at 25 degree centigrade.

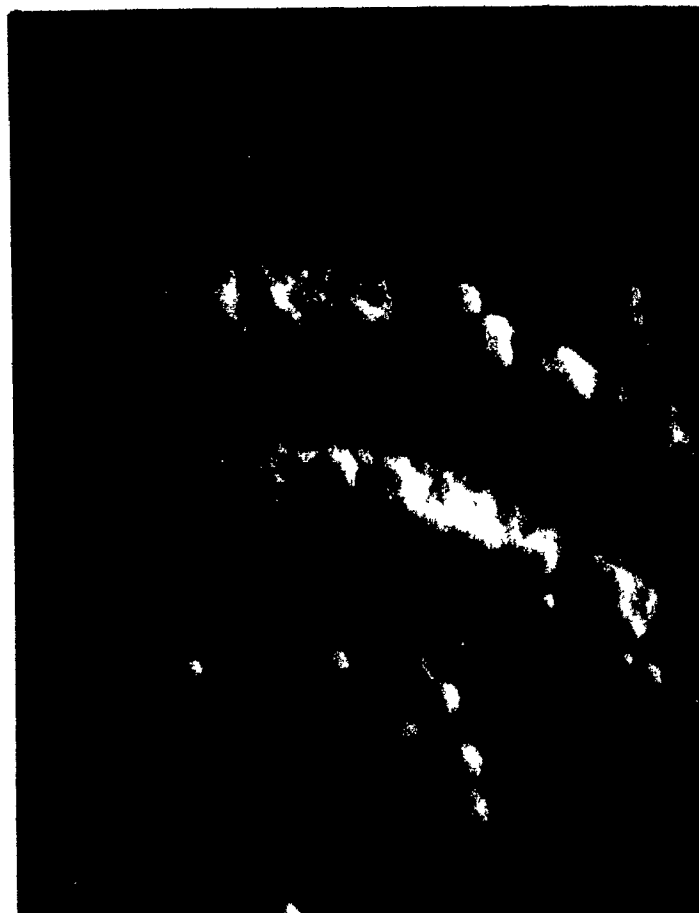


Plate 29. Zonation of the stilboid fungus. Mag. 1500X.

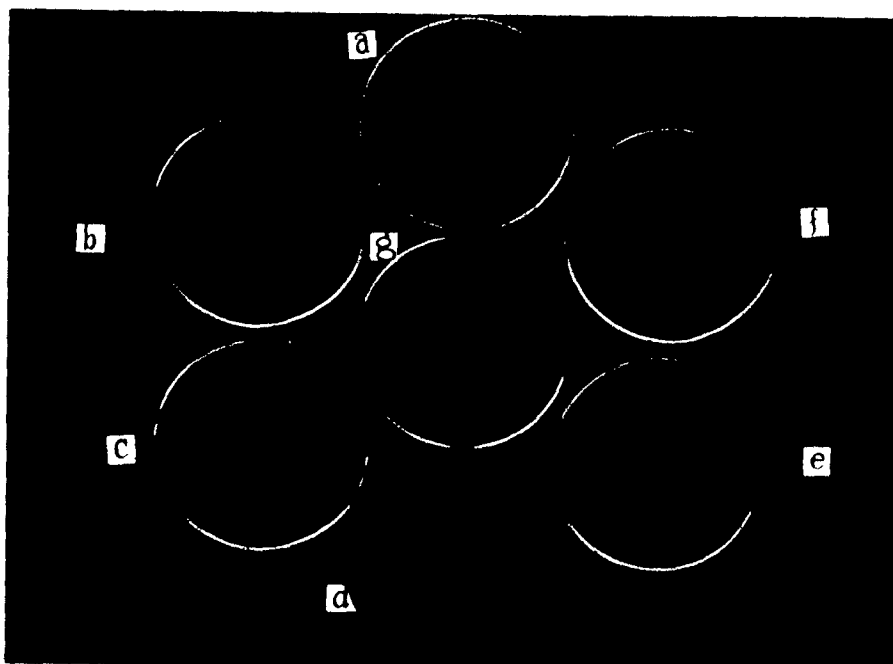


Plate 30

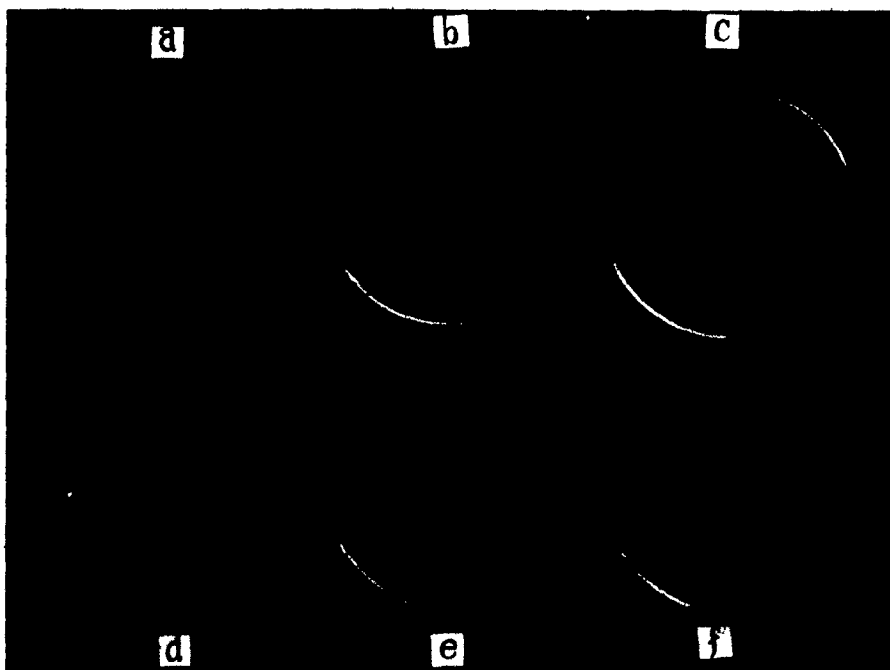


Plate 31

Cultures of S. zacalloxanthum grown on various media at 25° for 17 days.

Plate 30. In continuous darkness. a-PDA; b-CSA; c-LBA; d-MA; e-CMA; f-NA; g-MEA.

Plate 31. In alternating periods of light and dark, on same media as those in Plate 30 but without NA.

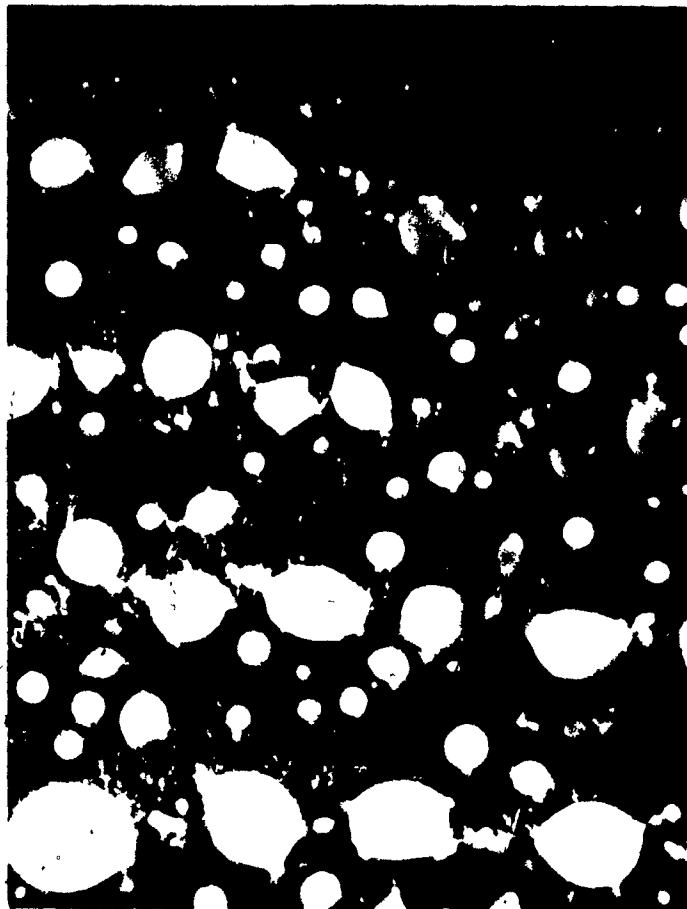


Plate 32. Stilbum zacalloxanthum Moore on potato dextrose agar showing the beginning of zonation made of spore laden synnemata. Mag. 1500X.

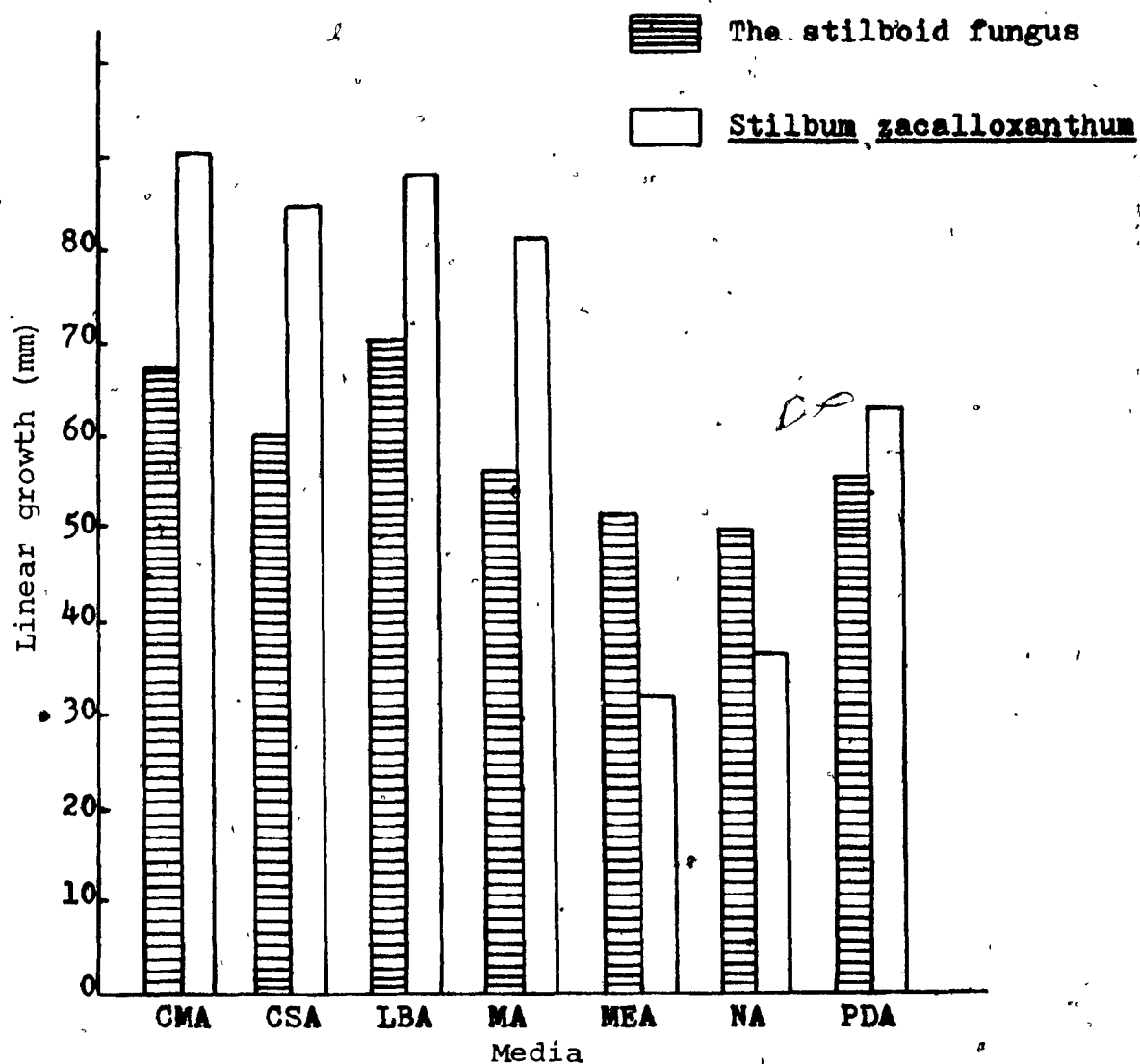


Figure 6. Colony diameters of fungi grown on seven kinds of nutrient media in continuous darkness for 17 days at 25°.

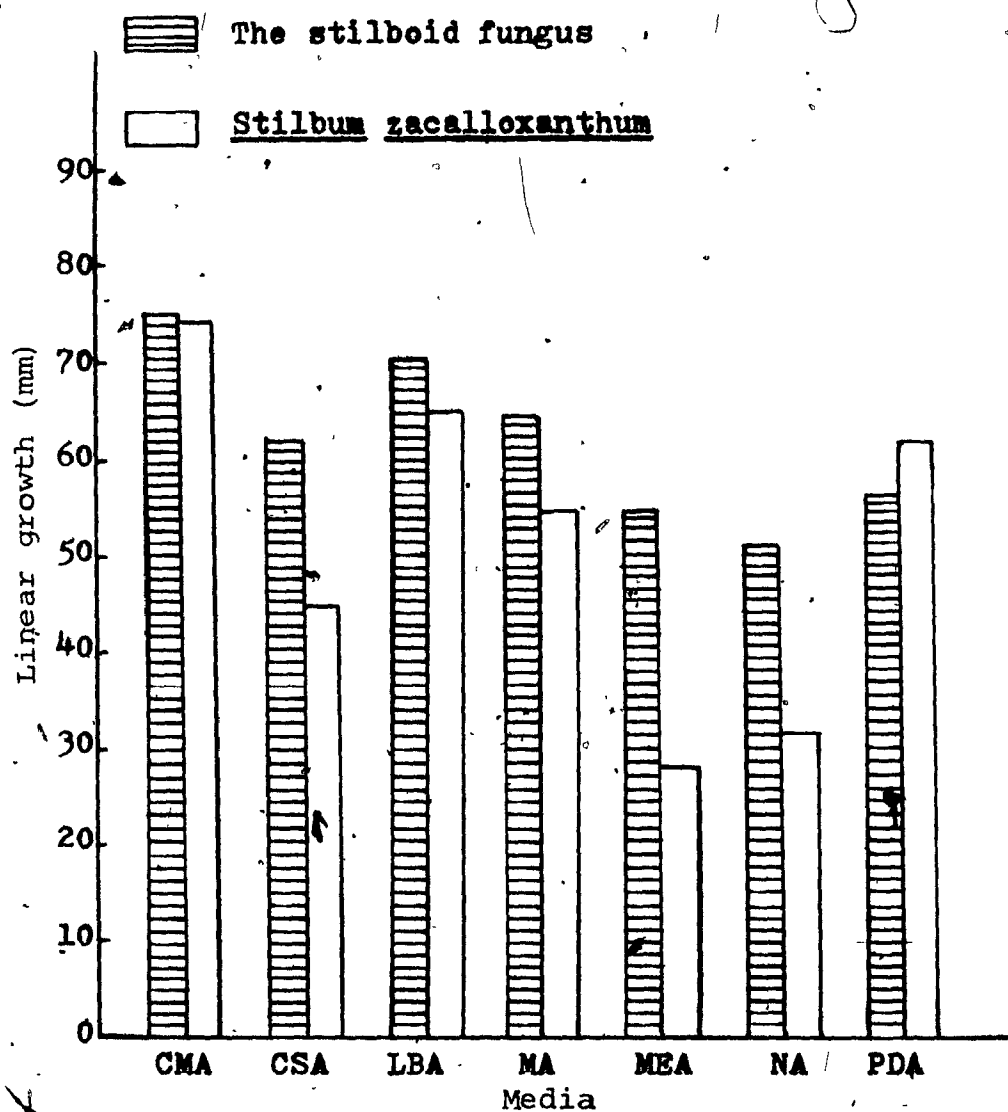


Figure 7. Colony diameters of fungi grown on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°.

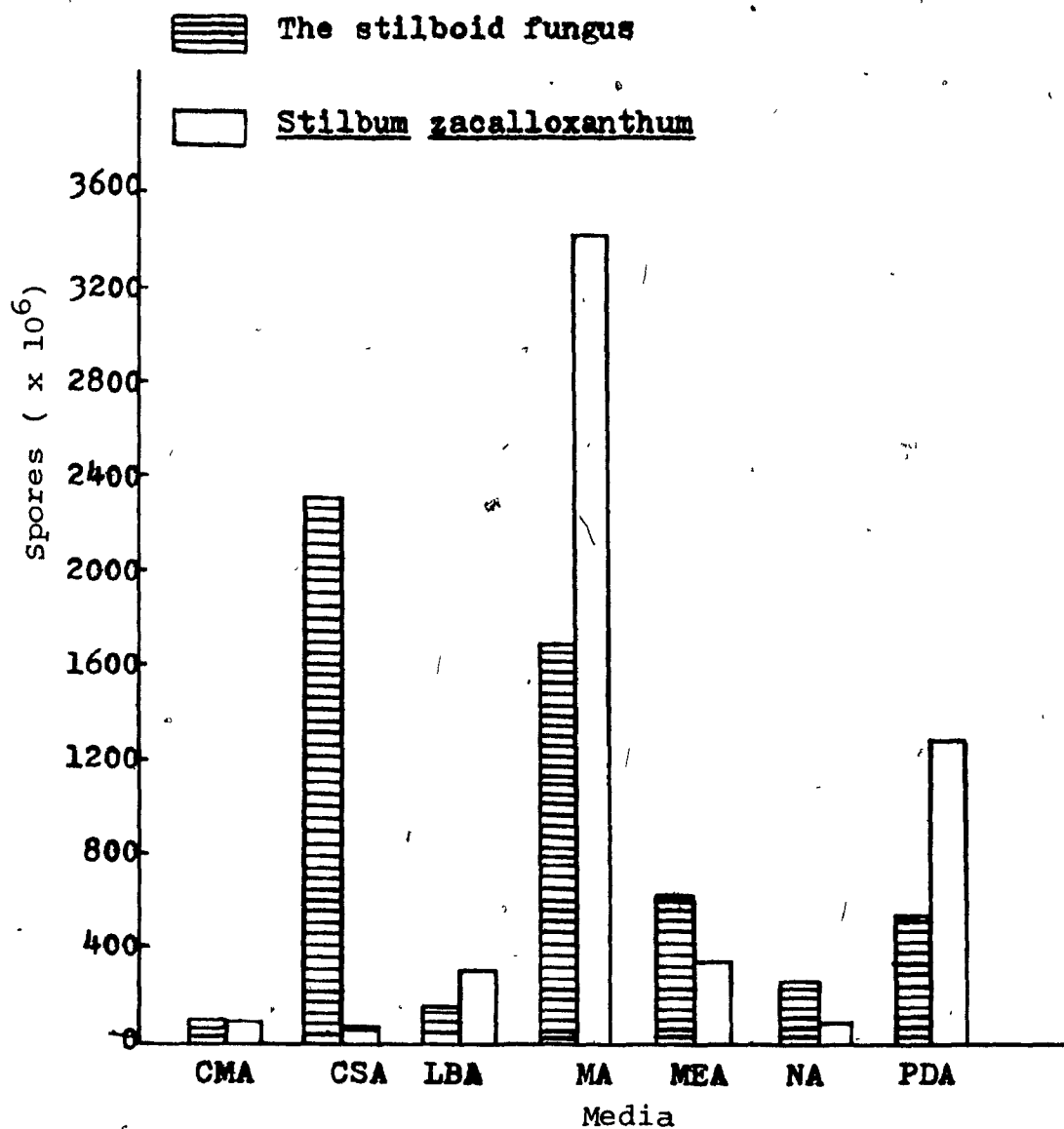


Figure 8. Sporulation of fungi grown on seven kinds of of nutrient media in continuous darkness for 17 days at 25°.

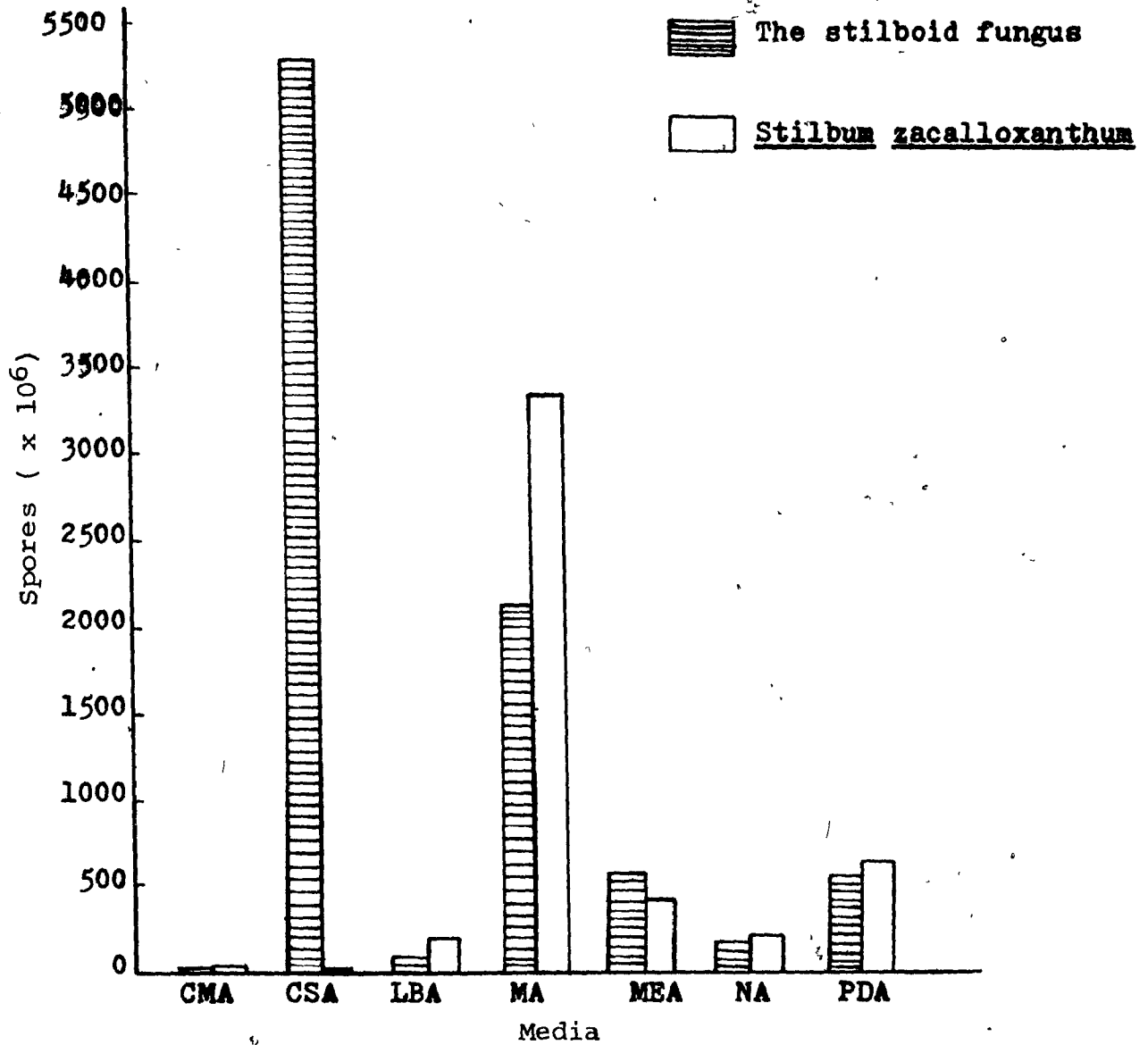


Figure 2. Sporulation of fungi grown on seven kinds of nutrient media in alternating periods of light and dark for 17 days at 25°.

Table 4. Growth rate of the single isolates of the stilboid fungus and Stilbum zacalloxanthum Moore on potato dextrose agar at room temperature with about twelve hours daily light period.

No. of day	The Stilboid Fungus*	<u>S. zacalloxanthum</u> *
	mm	mm
1	0	0
2	0	0
3	1	0
4	3	1
5	6	8
6	11	14
7	15	19
8	17	21
9	19	25
10	22	28
11	26	32
12	28	35
13	31	40

* Diameter of the colonies in millimeters.

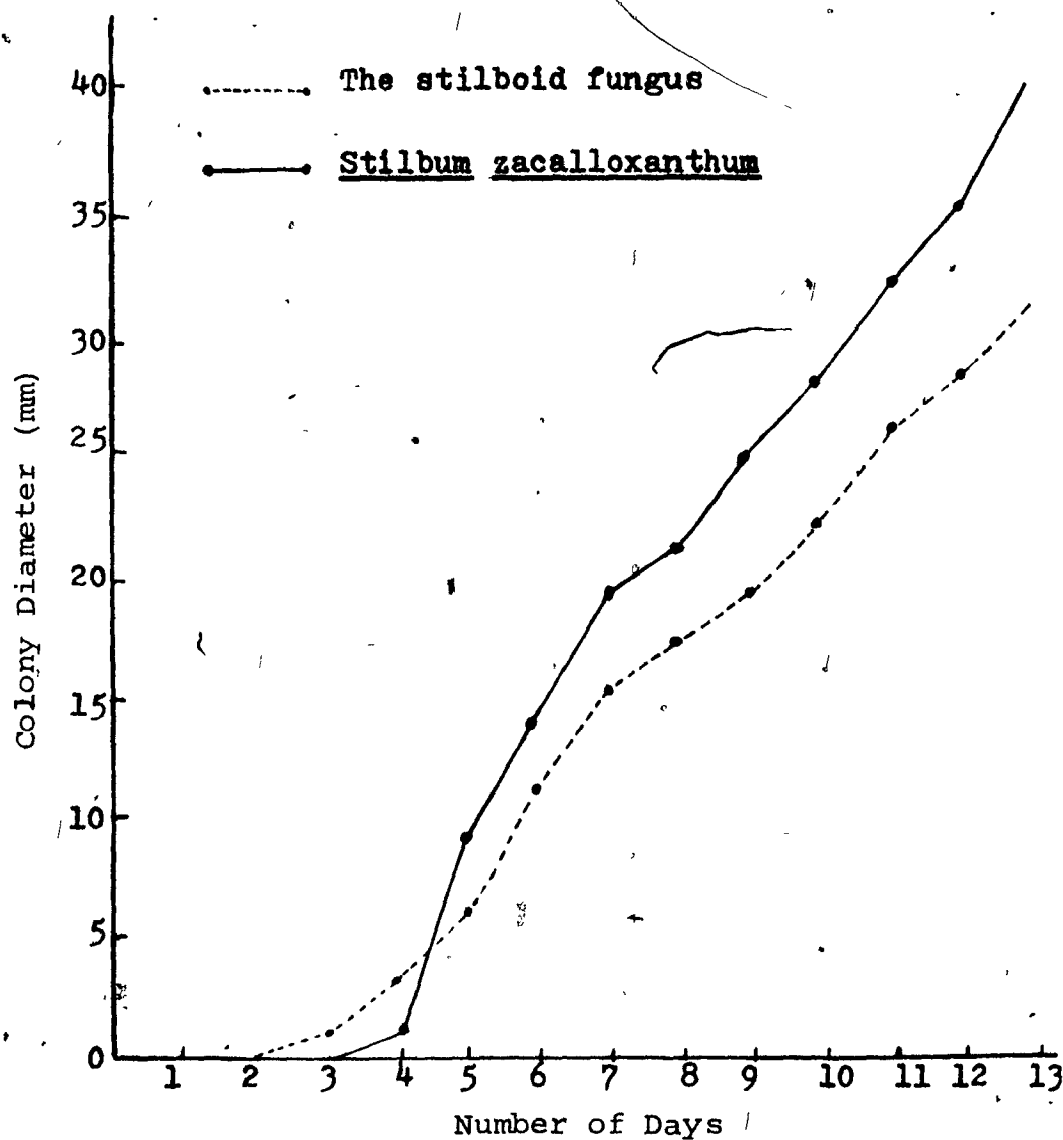


Figure 10. Comparative daily growth rate of fungi from single spore isolates, on PDA at room temperature for 13 days.

c. Growth Rate of the Two Stilboid Fungi

The growth rate was measured from a single isolated spore cultured on potato dextrose agar. The colony of the stilboid fungus could be detected by the naked eyes two days after isolation. The diameter was 1 mm by the fourth day. Stilbum zacalloxanthum could hardly be detected before the fifth day after isolation because of the initial hyaline nature of the colony, which later became bright yellow. After a slower start, S. zacalloxanthum grew faster than the unnamed stilboid fungus. The diameter of S. zacalloxanthum was 9 mm larger than that of the unnamed stilboid fungus by the thirteenth day.

Table 5. The effect of the initial pH of potato dextrose agar media on the growth of the stilboid fungus for 10 days at 20 degree centigrade.

pH of medium	Linear mycelial growth*
2.5	0
3.0	7
4.0	14
4.5	17
5.6	33
6.5	22
8.0	29
9.5	25
10.5	21
11.0	20
11.5	16

* Diameter of the colonies in millimeters.

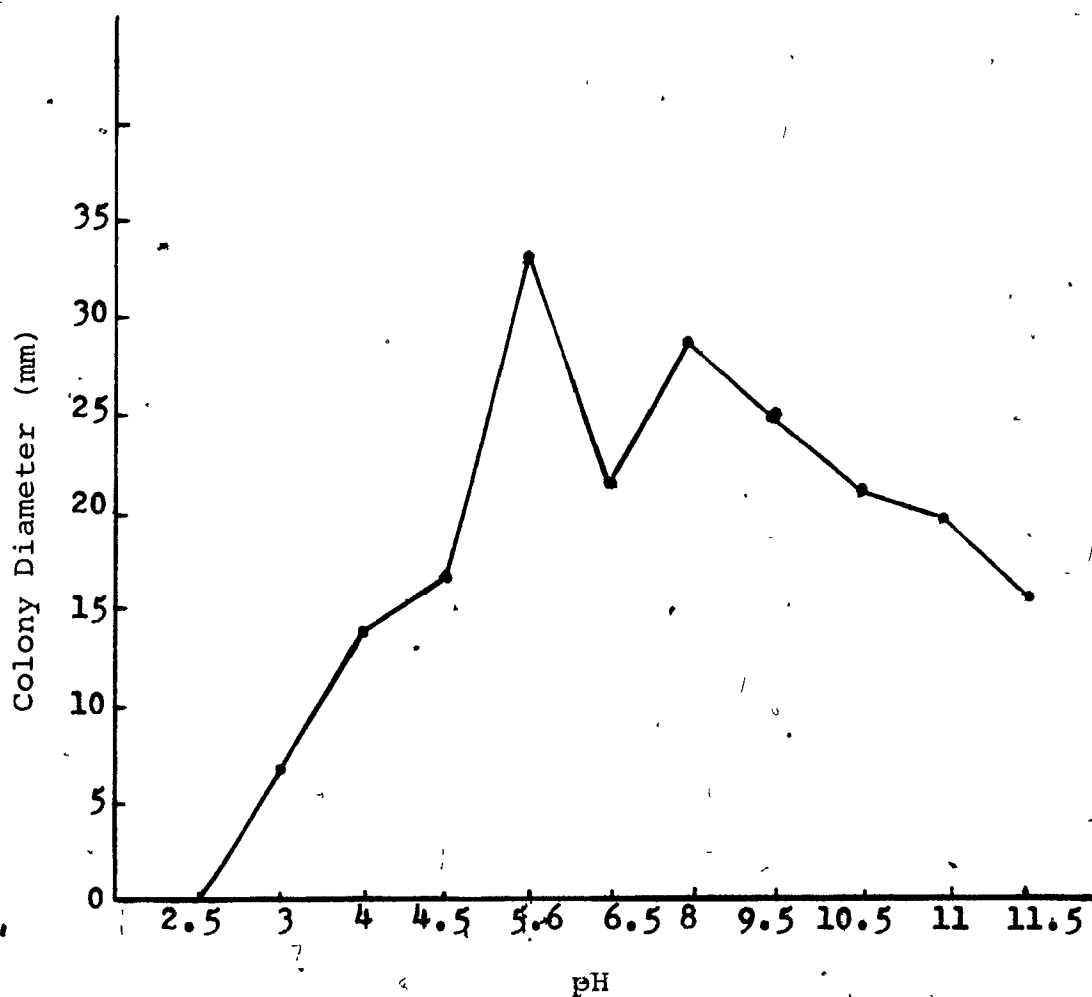


Figure 11. Effect of the initial pH of potato dextrose agar media on the growth of the stilboid fungus for 10 days at 20 degree centigrade.

Table 6. The effects of continuous light, continuous darkness and alternating periods of light and dark on colony diameter and sporulation of single spore isolates of the stilboid fungus on potato dextrose agar for 18 days at 20 degree centigrade.

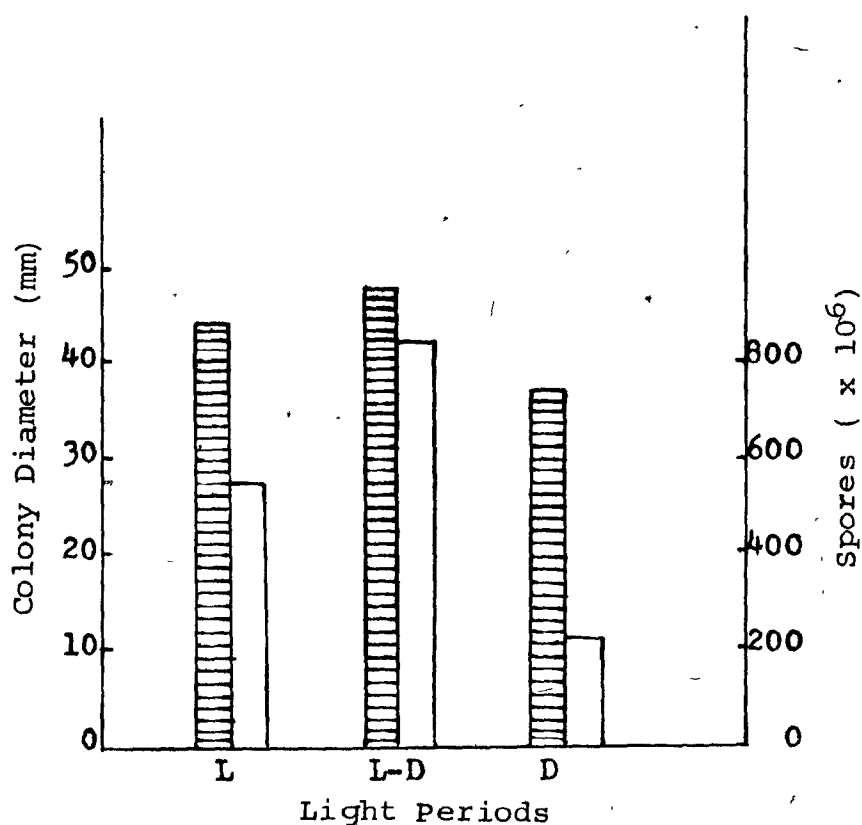
Continuous light*		Alt. light-dark**		Continuous darkness	
Mycelial growth***	Spore per colony $\times 10^6$	Mycelial growth	Spore per colony $\times 10^6$	Mycelial growth	Spore per colony $\times 10^6$
44	575	48****	843	37	238

* Light intensity of 15,000 ft-candles.

** 12-hr light at 15,000 ft-candles followed by 12-hr darkness.

*** Average diameter of four colonies in millimeters.

**** Zonation occurred in all colonies.



Diameter of colony



Number of spores per colony

L --- Continuous light

L-D --- 12-hr light, 12-hr dark

D --- Continuous darkness

Figure 12. Growth and sporulation of the stilboid fungus in continuous light, continuous darkness and alternating periods of light and dark on potato dextrose agar for 18 days at 20°.

Table 7. Growth rate of the stilboid fungus in continuous light, continuous darkness, and alternating periods of light and dark on potato dextrose agar at 20°.

Day	Colony diameter ^a		
	Cont. light ^b	Alt. light-dark ^c	Cont. darkness
	mm	mm	mm
7	21	20 ^d	16
10	31	30	26
15	46	47	40
18	52	54	48

^a Average diameter of four colonies in millimeters.

^b Light intensity of 15,000 ft-candles.

^c 12-hr light at 15,000 ft-candles followed by 12-hr darkness.

^d Zonation occurred in all colonies.

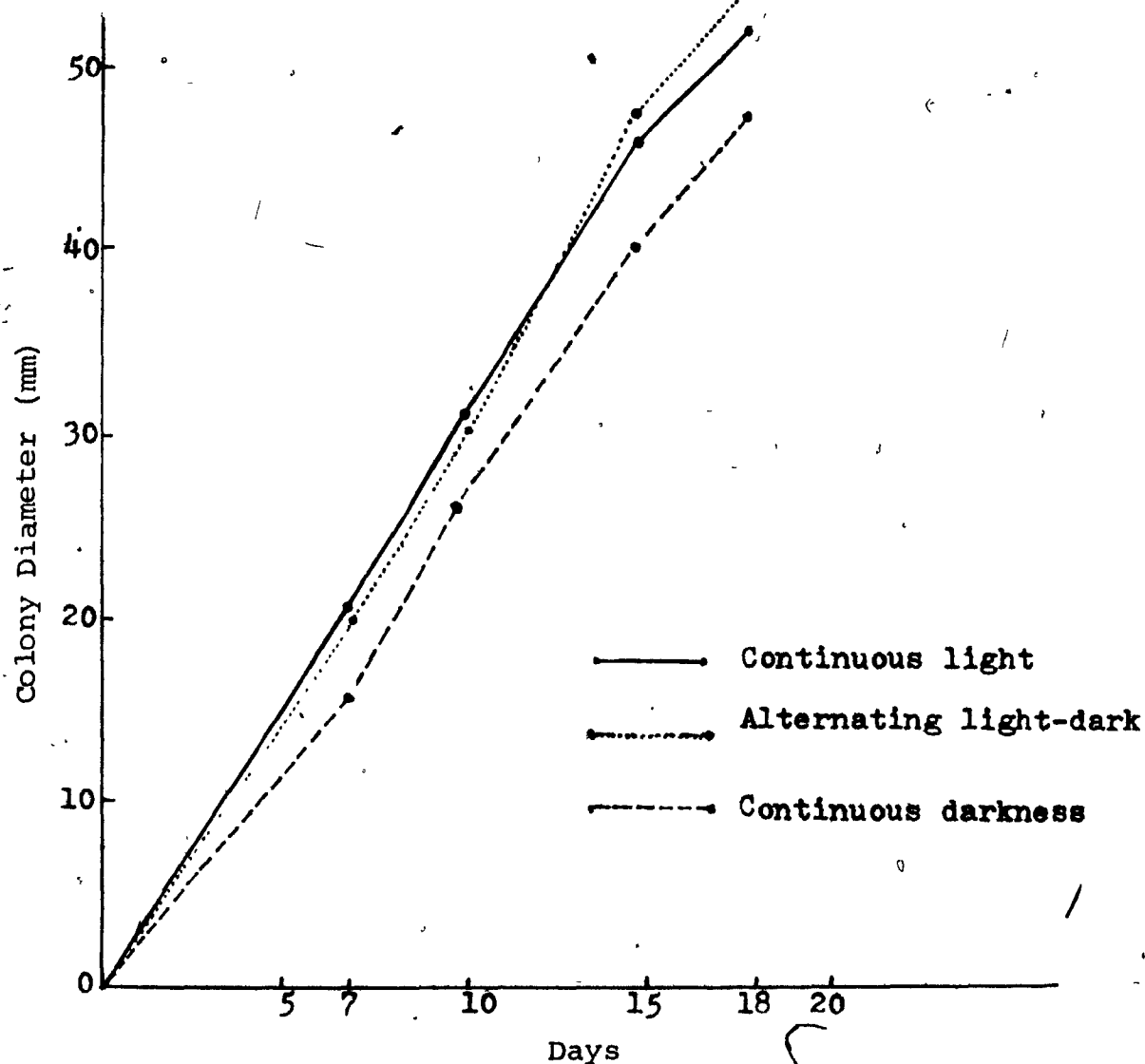


Figure 13. Growth rate of the stilboid fungus in continuous light, continuous darkness and alternating periods of light and dark, on potato dextrose agar for 18 days at 20°.

3. pH of a Medium

Under the experimental conditions of these tests, the optimum initial pH of potato dextrose agar for growth of the stilboid fungus was 5.6 (Table 5 and Figure 11). It seems that the most suitable pH next to 5.6 was at 8.0. The fungus grew better between the range of pH from 5.6 to 11.0 than at any pH value lower than 4.5. It is interesting that growth was retarded at pH 6.5 relative to either pH 5.6 or 8.0. To make certain that this was true the experiment had repeated twice with 4 replicates for each pH level each time. The results were similar in all repetitions of the test. Dual pH optima are not unusual with unbuffered culture media (Cochrane, 1958).

4. Light

Light has a marked effect on many fungi. This effect may be divided into two categories: morphogenetic effect and non-morphogenetic effect (Page, 1965). A review of the literature on certain effects of light on fungi, such as, reproduction, morphology, pigmentation, and phototropic phenomena may be found in the extensive bibliography of Marsh et al (1959).

The purpose of this study was to determine some of the effects of light on the stilboid fungus. The fungus was exposed to different light periods and its growth and sporu-

lation were determined. The following statements describe the effects of light on certain features of the fungus.

The stilboid fungus grown in all controlled light periods under these experimental conditions showed that the effect of the light did not cause any morphological change although it influenced the rate of growth and sporulation. Because the synnemata are positively phototropic, they bent toward the source of light whenever it was unidirectional, from one side. This is shown in Plate 33.

When the stilboid fungus was grown in alternating light-dark periods of 12-hr light at 15,000 ft-candles followed by 12-hr dark, on potato dextrose agar, for 18 days at 20°, zonation corresponding to the periods of light occurred in all cultures. Alternating light-dark periods had a similar zone-inducing effect on cultures of S. zacalloxanthum, with 13 rings being formed in as many light periods. (Plate 36). The culture of S. zacalloxanthum grown in continuous darkness produced more conidia than did those grown in either continuous light or in alternating periods of light and darkness. The zonation was absent in all cultures grown on this medium in continuous light or darkness. Spore production and mycelial growth in diurnal cycles of light and dark was always greater than in continuous light or darkness at 20°. Table 6 and Figure 12 show the growth and sporulation under these three light conditions.

The growth rate at 20° in continuous and alternating

light-dark periods is given in Table 7 and Figure 13. Between the seventh and tenth day, growth of the fungus in continuous light was faster than those in either alternating light-dark periods or in continuous darkness. On the tenth day cultures in alternating light-dark were larger in diameter than those in continuous light or dark, and by the eighteenth day they were 3 mm larger. The cultures in continuous light were of a brighter yellow colour than those in alternating light-dark. Cultures in continuous darkness were creamy in colour. It is believed that light stimulates growth, spore and carotenoid production in many fungi (Carlile, 1965).

It is clear that alternating light-dark periods had a marked effect on zonation of the stilboid fungus. The first zone or ring became distinctly visible usually after 4 to 5 days, and one ring was produced daily thereafter. When subjected to an alternating schedule of 24-hr light and followed by 24-hr dark for 18 days (Plate 34), the fungus produced zones that were 2 millimeters apart. One zone was produced for each every 24 hour period of light, except for the last two or three had not yet been produced. After 18 days, there were 6 zones of synnemata in the cultures of 24-hr light - 24-hr dark while there were at least 12 distinct zone in the cultures of of 12-hr light - 12-hr dark periods. When the cultures were exposed to light for only five minutes in every five days for 22 days, the fungus showed four rings, five millimeters apart, and corresponding to the periods of five minutes

exposure to light (Plate 35, left). When cultures were kept in continuous light for five days and then transferred into the dark for five minutes, in a schedule and on media similar to the above, they did not show any evidence of zonation (Plate 35, right).

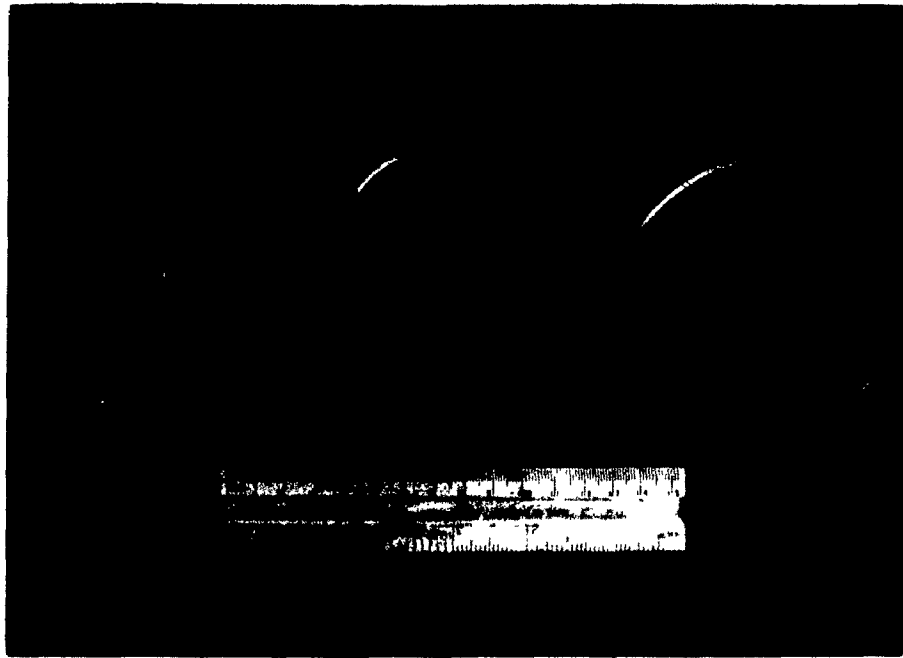


Plate 33. Cultures of the stilboid fungus grown at 20° in (left) continuous darkness, (center) alternating light-dark, and (right) continuous light, on potato dextrose agar for 18 days.

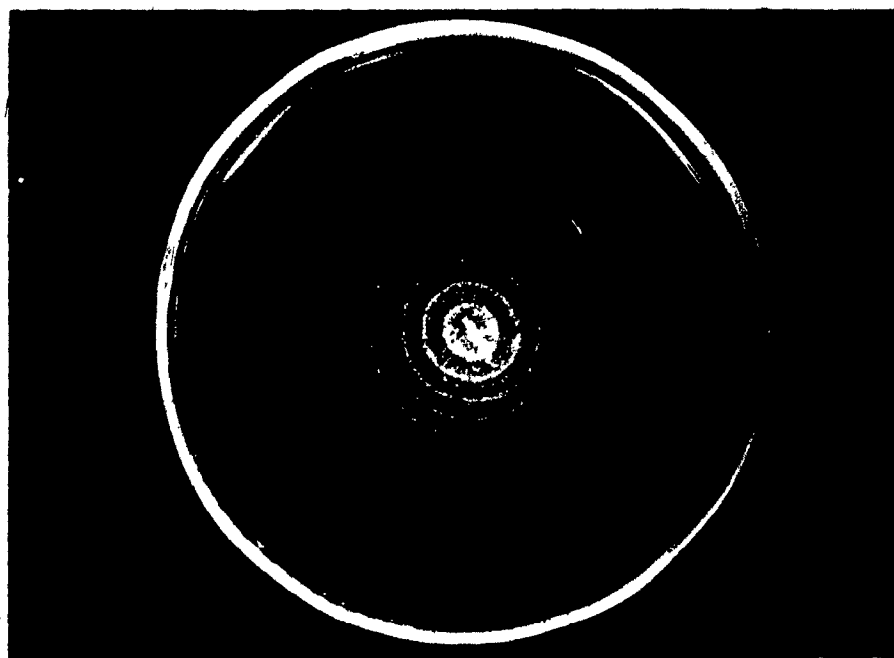


Plate 34. A culture of the stilboid fungus grown at 20° in alternating light-dark periods, each of 24 hours duration on potato dextrose agar for 18 days.

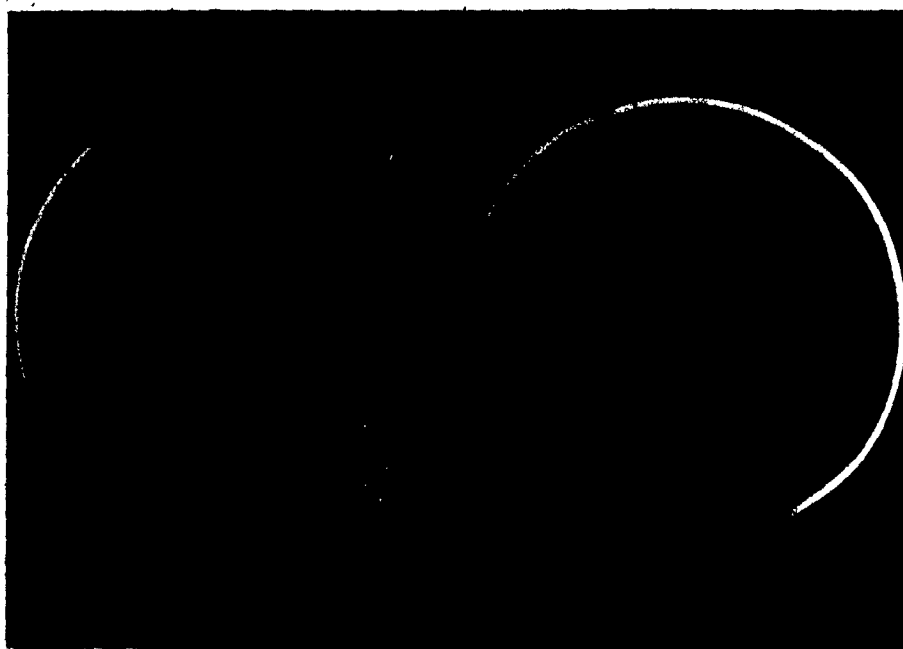


Plate 35. Cultures of the stilboid fungus grown at 20° on potato dextrose agar for 22 days in alternating light-dark period schedules of; (right) 5-days light - 5-min. dark, and (left) 5-days dark - 5-min. light.

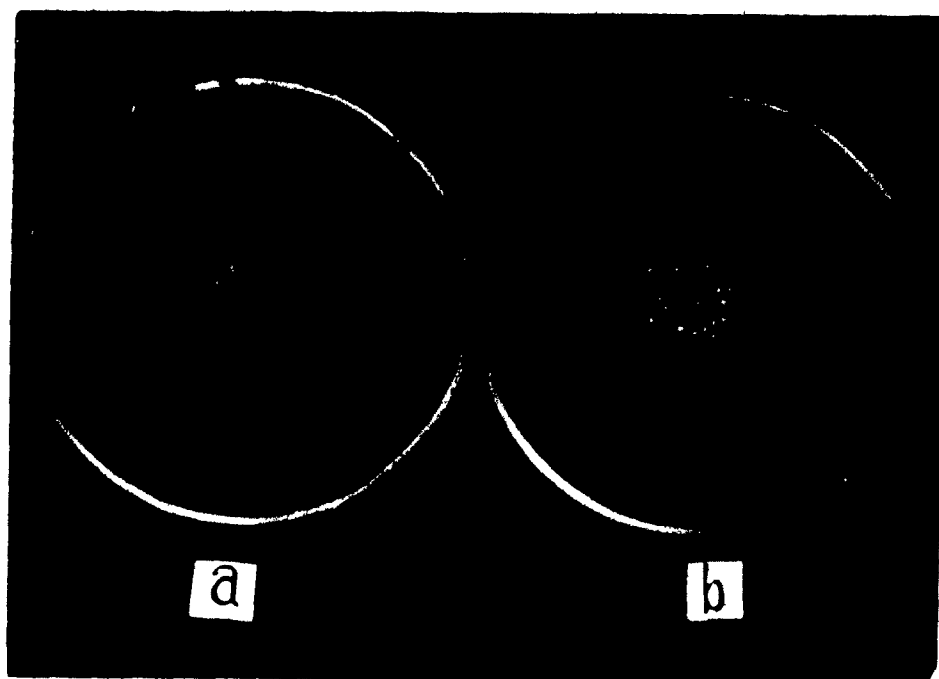


Plate 36. Cultures of Stilbum zacalloxanthum Moore grown for 17 days at 25° on potato dextrose agar, (a) in alternating light-dark schedule of 12-hr light - 12-hr dark, and (b) in continuous darkness.

C. COMPETITION BETWEEN THE STILBOID FUNGUS AND OTHER FUNGI

This experiment was done in order to learn something of the competitive saprophytic ability of the stilboid fungus with the various fungi tested.

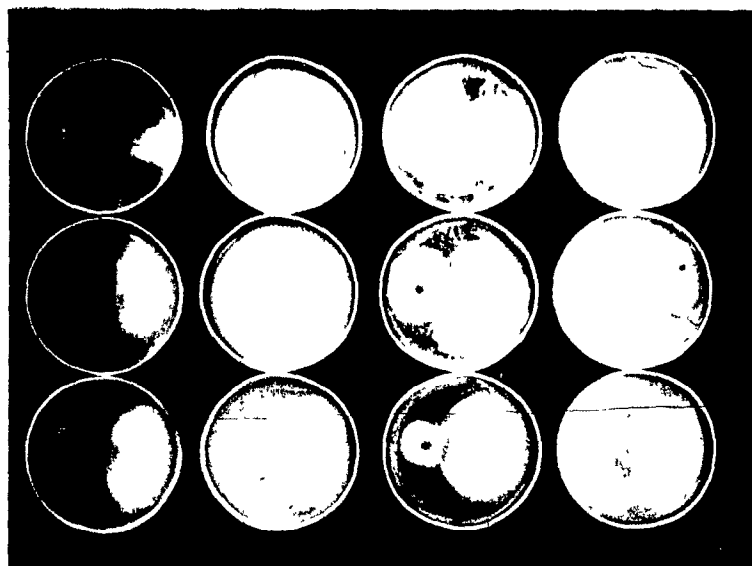
Plate 37 (a) to (e) show that the stilboid fungus was very weak when it was growing with, (k) Mucor hiemalis; (l) Oospora sp.; (o) Sclerotium rolfsii; (p) Sordaria sp.; and (s) Trichoderma sp. Regardless of whether the above named fungi were transferred to the culture media before, at the same time or after the stilboid fungus it was totally covered by them.

When the stilboid fungus was cultured with, (a) Arthrobotrys superba; (d) Botrytis sp.; (e) Chaetomium sp.; and (g) Fusarium oxysporum, there was evidence of growth inhibition as a distinctive zone occurred at the area of contact. Later, these fungi also grew over the colony of stilboid fungus, and the distinctive zone was also covered by them. Growth of the stilboid fungus underneath did not appear to have been greatly inhibited. The colony of Botrytis sp. grew surrounding the stilboid fungus colony which was inoculated later or at the same time.

In cultures of the stilboid fungus grown with the following fungi: (c) Asperigillus repens; (f) Diplodia zeae; (h) Gliocladium roseum; (i) Gliomastrix sp.; (j) Helminthosporium sativum; (m) Penicillium expansum; (n) Phoma sp.;

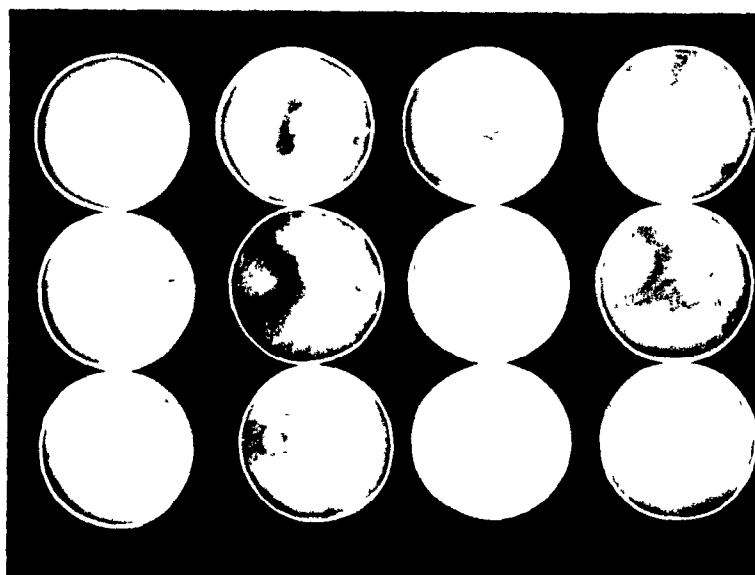
(q) Stachybotrys atra; (r) Stilbum zacalloxanthum; and Verticillium dahliae, there was an evidence of inhibition as area of separation sometimes occurred between colonies of each of the above fungi and the stilboid fungus. The inhibition was more evident when it grew with (j) Helminthosporium sativum and (q) Stachybotrys atra. Inhibition between the stilboid fungus and (i) Gliomastrix sp. and (n) Phoma sp. resulted in an alteration in the colour of the cultures in the area around the contact zone.

The competitive saprophytic ability of the fungi tested in this experiment was usually greater than that of the stilboid fungus. The stilboid fungus may have been weaker because of its slower rate of growth, its lack of ability to produce some inhibitory toxin or metabolic product or a combination of these. However, it did show evidence of an ability to tolerate the toxin or metabolites products from other competing fungi. This characteristic of the stilboid fungus may be largely responsible for its survival in nature. The author has never found it growing naturally unless competing fungi were dead or otherwise inactive when the isolation was made.



a b c d

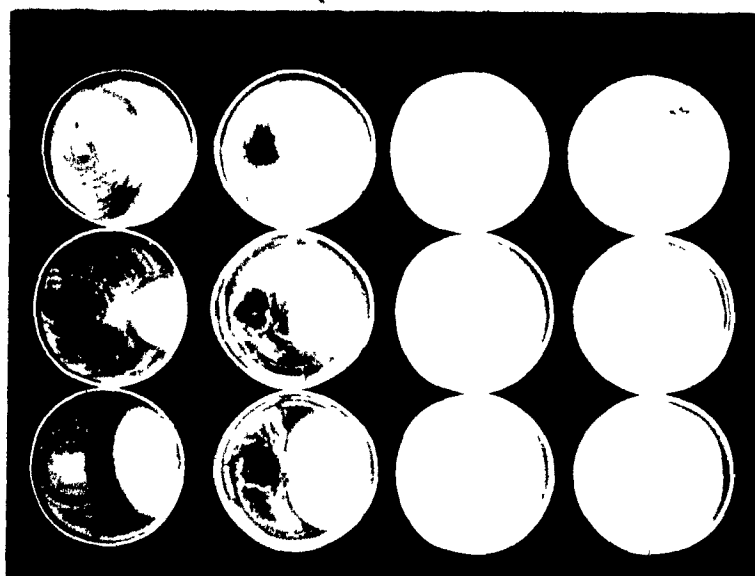
Plate 37 a



e f g h

Plate 37 b

Plate 37 a and b. The stilboid fungus growing with:
 (a) Alternaria sp.; (b) Arthrobotrys superba;
 (c) Aspergillus repens; (d) Botrytis sp.;
 (e) Chaetomium sp.; (f) Diplodia zeae;
 (g) Fusarium oxysporum; (h) Gliocladium roseum.



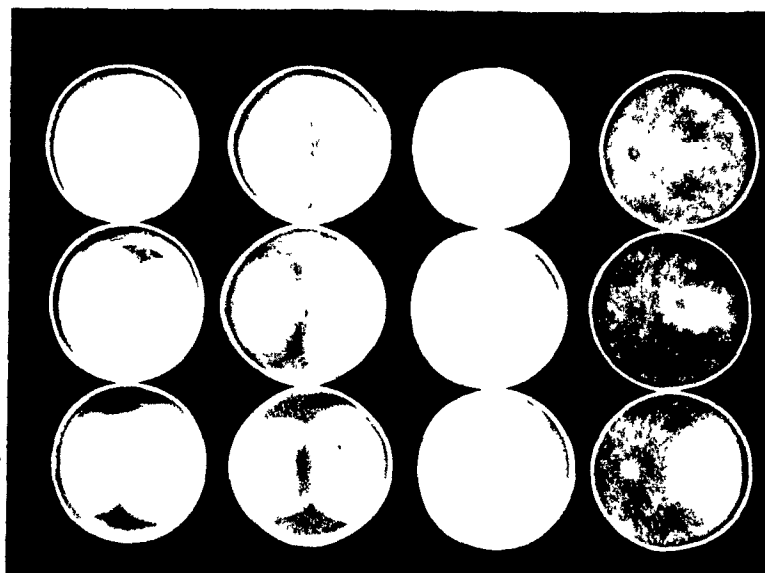
i

j

k

l

Plate 37 c



m

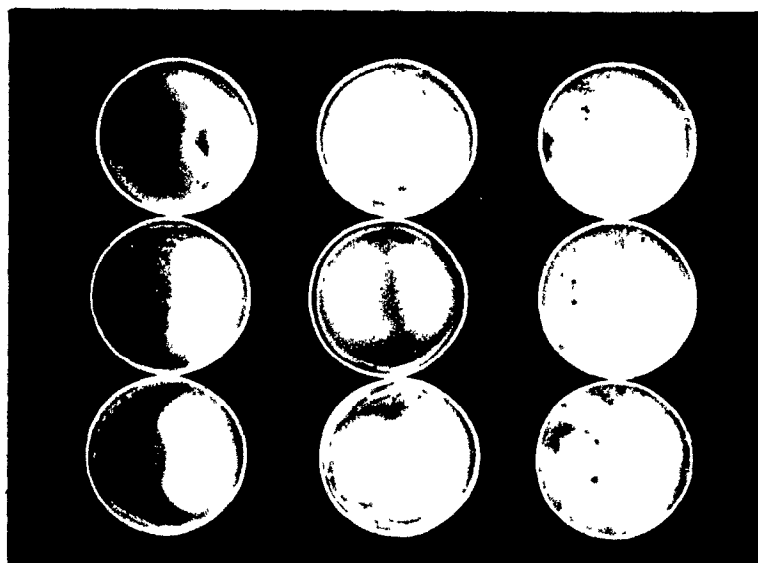
n

o

p

Plate 37 d

Plate 37 c and d. The stilboid fungus growing with:
 (i) Gliomastrix sp.; (j) Helminthosporium
sativum; (k) Mucor hiemalis; (l) Oospora sp.;
 (m) Penicillium expansum; (n) Phoma sp.;
 (o) Sclerotium rolfsii; (p) Sordaria sp.



q

r

s

Plate 37 e. The stilboid fungus growing with:
(q) Stachybotrys atra; (r) Stilbum zacalloxanthum;
(s) Trichoderma sp.

D. MORPHOLOGICAL ABNORMALITIES OF THE STILBOID
FUNGUS INDUCED BY VOLATILE
MICROBIAL METABOLITES

(1) Under the experimental conditions used, the control culture of the stilboid fungus produced hyphae of about 1.4 to 2.2 micrometers wide with smooth mycelial walls. The culture grown with Agrobacterium tumefaciens had abnormal hyphae which stained dark blue with cotton blue in lactophenol. The hyphal tips commonly became narrower or, less commonly, bigger than the normal one (Plate 38 a,b,c, and d). Vesicles of approximately 10 micrometers in diameter were produced intercalarily, terminally and laterally (Plate 38 a, d, and e). Some of these vesicles were empty, only the remaining thin walls being visible. Some of them may have collapsed when fragments of the cultures were mounted with cotton blue in lactophenol, as suggested by Moore-Landecker and Stotzky (1973). Other morphological features usually remained normal.

Similar effects occurred in cultures grown with Rhizobium leguminosarum. The abnormally large hyphae were, in some instances, even larger than those found in the cultures with Agrobacterium tumefaciens. Vesicles, up to 15 micrometers, in diameter were produced intercalarily, terminally, and laterally (Plate 39 a,b,c, and f). Some branches of the hyphae were narrow at the beginning but became widened and extended, then tapered to narrow tips. The hyphal tips often

branched equally in two, as illustrated by (e) in Plate 39. This is an unusual feature for any normal vegetative hyphae. The cell walls of the hyphae were often rough and uneven, relative to normal hyphae. A peculiar fan-like structure was observed (Plate 39 g). Some sections of the vesicles and mycelia were empty. These abnormal structures always stained dark blue with cotton blue in lactophenol, and were thus very distinct from the normal structures.

Except for an absence of vesicles, many of the morphological abnormalities of the stilboid fungus which occurred in cultures grown with Bacillus cereus were similar to those that occurred with A. tumefaciens and R. leguminosarum. There was however more evidence of branched hyphal tips, the hyphae sometimes branching two to three times (Plate 40 b and c). Parts of the hyphae swelled up to 10 micrometers wide, specially in the area of branching hyphae (Plate 40 a, d, and e). Empty cells were also found, as indicated by (a). Swollen intercalary cells were often shorter than the other cells (Plate 40 f). Narrow tapering hyphal tips were also observed.

(2) One culture of each of the three bacteria mentioned above were grown side by side with the stilboid fungus on nutrient agar is shown in Plate 41. The cultures of A. tumefaciens and B. cereus, which grew faster than R. leguminosarum, spread over the colonies of the stilboid fungus (Plate 41 a and b). There was little, if any, evidence of inhibition between these bacteria and the fungus. In the cultures grown

with R. leguminosarum, the stilboid fungus grew normally at the beginning but later showed evidence of some inhibition. The fungus grew around the colony of R. leguminosarum neither organism grew over the other. One example of this growth is shown in Plate 41 c. Plate 42 shows the growth habit of the stilboid fungus alone on nutrient agar.

In summary, the volatile products from the bacteria tested produced morphological changes in the stilboid fungus. The abnormalities were the formation of vesicles, hyphal distortion and excessive branching. It is known that these alterations have been induced in other fungi by potential inhibitory or toxic conditions (Barnett & Lilly, 1966; and McQuade, 1963); antibiotics (Aytoun, 1956; Barathova et al, 1969; Links et al, 1957; and Napier et al, 1956); toxic chemicals (Bitting, 1920; Childs et al, 1971; Vercourt, 1952 and Vincent, 1947) or staling substances in the medium (Callen, 1940; Elarosi, 1957; and Park & Robinson, 1964); growth in liquid culture (Duckworth & Harris, 1949; and Ling-young, 1930); exposure to volatile chemicals (Sansome, 1946; and Strzelczyk, 1968); volatiles from plant materials (Fries, 1973). The thickened walls described by Moore-Landecker & Stotzky (1973) were not observed in the stilboid fungus. Conidiophores and conidia were usually normal. The abnormalities seen in this experiment were the same in spot, streak and film cultures of the three species of bacteria tested.

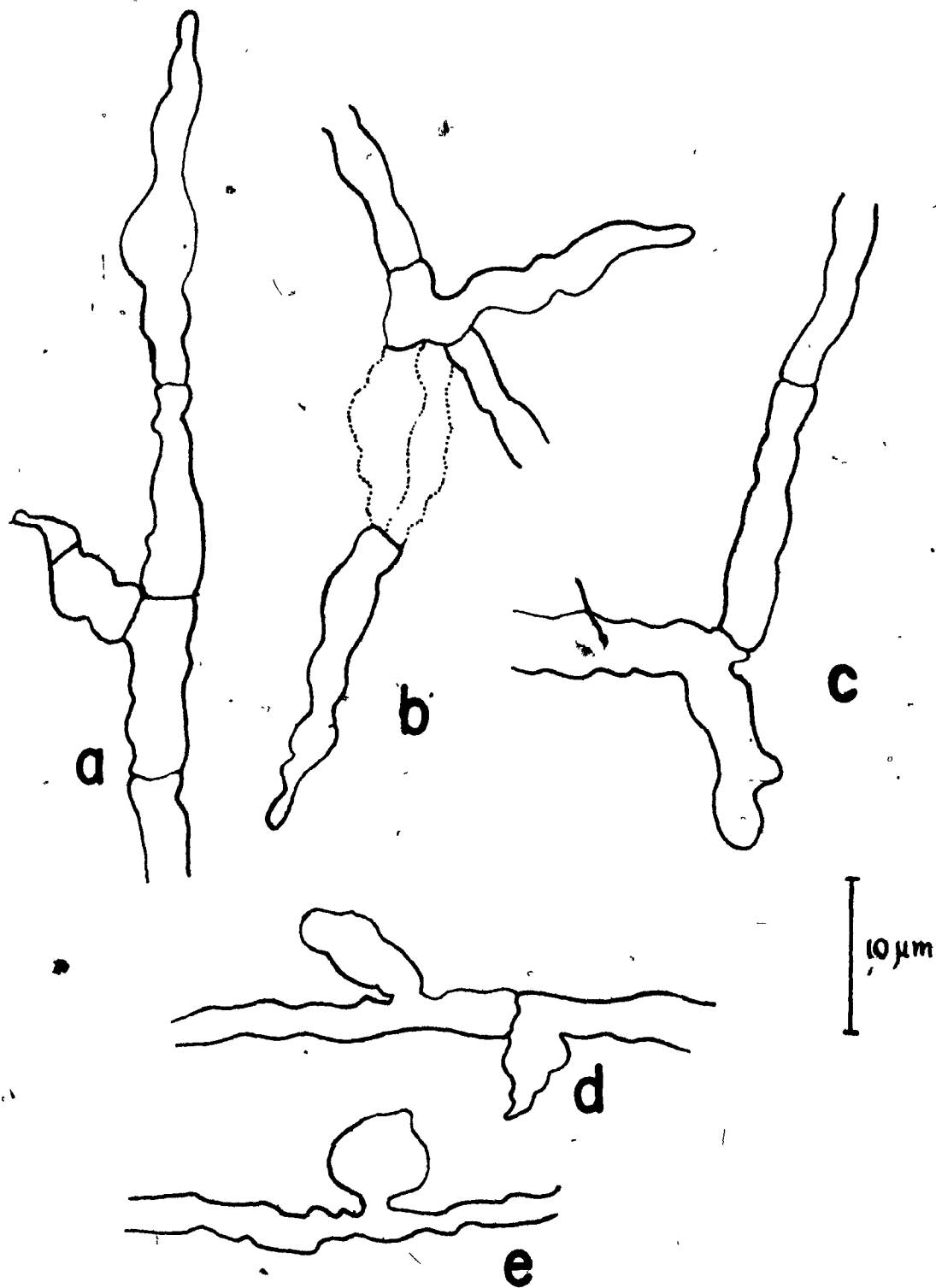


Plate 38. Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by Agrobacterium tumefaciens. (a) Abnormal hyphal tip; (b) the empty cell of the hypha; (c) swollen hypha; and (d & e) vesicles formed laterally.

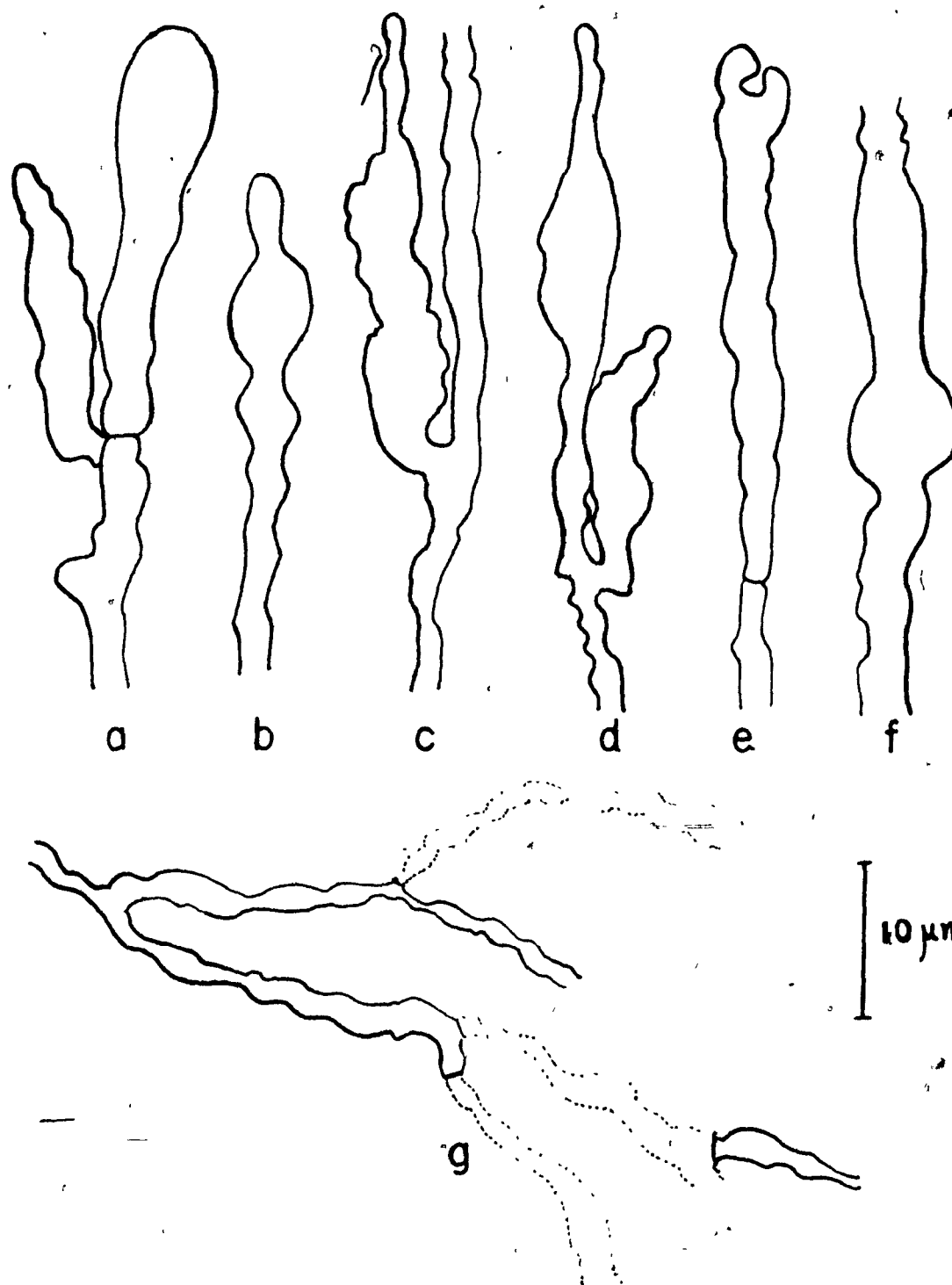


Plate 39. Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by Rhizobium leguminosarum. (a) Vesicle formed terminally; (b,d,f) vesicles formed intercalary; (c) vesicle formed laterally; (e) branched hyphal tip; and (g) fan-like structure of hypha.

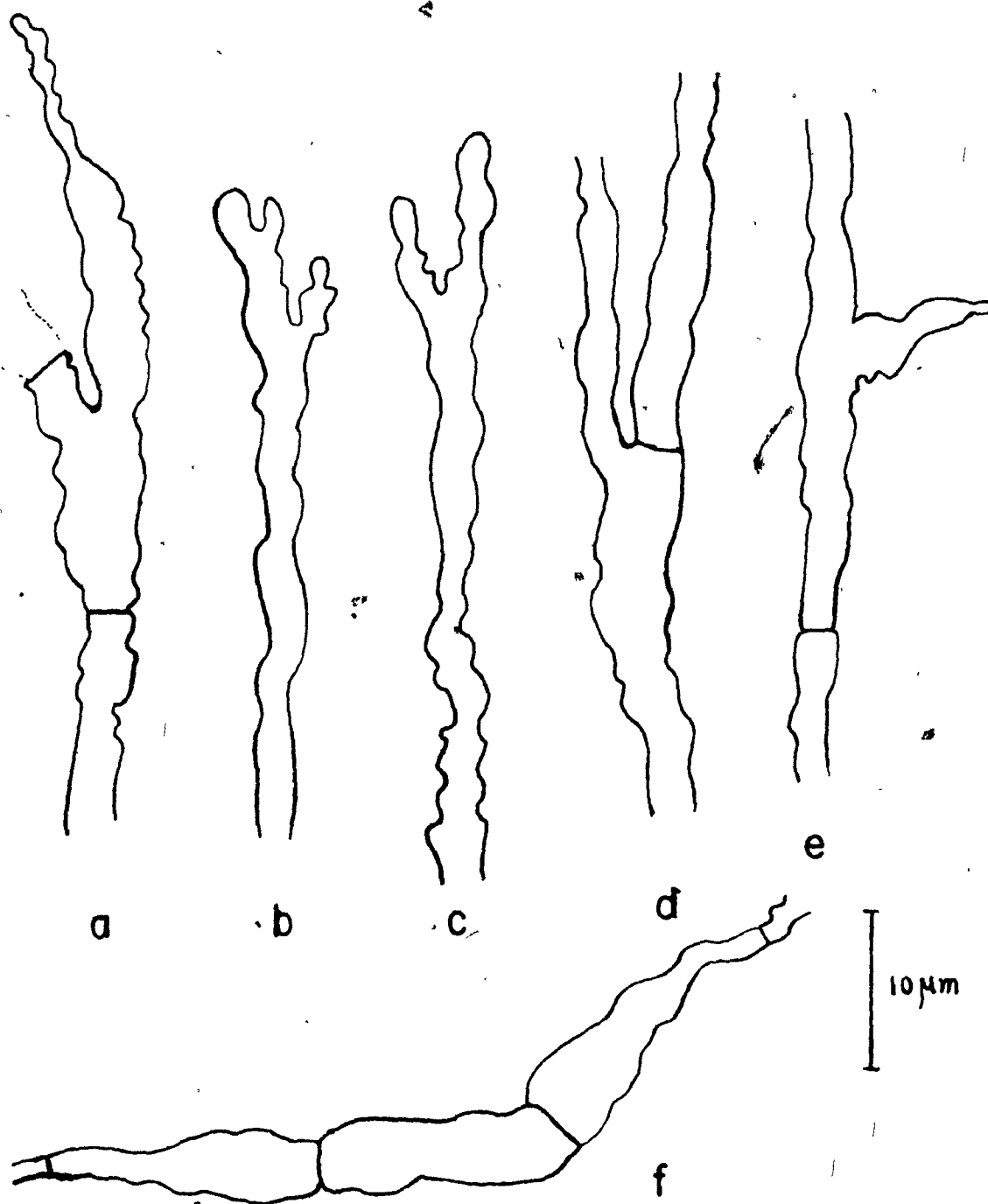


Plate 40. Camera lucida drawings of hyphal abnormalities in the stilboid fungus induced by Bacillus cereus. (a) Swollen hypha with an empty cell; (b & c) Branched hyphal tip; (d) Swollen hypha; (e) Narrow hyphal tip; and (f) Short swollen cell of the hypha.

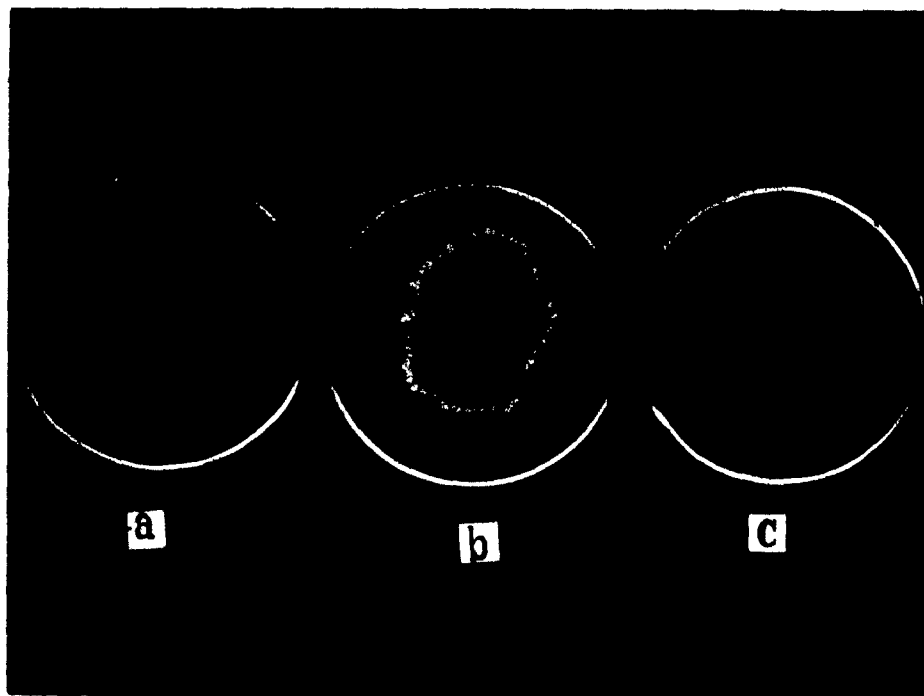


Plate 41. Cultures of the stilboid fungus grown with (a) Agrobacterium tumefaciens; (b) Bacillus cereus; and (c) Rhizobium leguminosarum, on nutrient agar for 7 days at room temperature.



Plate 42. 7-day culture of the stilboid fungus on nutrient agar at room temperature.

E. SURVIVAL IN SOIL

Only five synnemata of the stilboid fungus were produced from the inoculated soil of this experiment after 30 days. Instead of multiplying they disappeared from view after 2 to 3 months. When some of this soil was plated on PDA the fungus reappeared, indicating that it was surviving in the soil, probably in the form of spores. At first, the synnemata from soil were different from the usual ones as they were dry and hard. Then later they gave rise to the slimy synnemata. These synnemata appeared to be similar in all respects to those that grew from plants.

The author failed to isolate the fungus from natural uninoculated soil whether it did or did not contain plant materials. However, it was isolated from inoculated sterile soil used for growing clover plants in another experiment. In this instance there was evidence that the fungus was growing on plant materials or debris contained in the soil rather than in or among soil particles alone. When pieces of this debris were isolated in a Petri dish containing moist filter paper, the fungus produced a large number of synnemata on them which survived for more than six months. They grew fast and usually in group of 5 or more synnemata. The heads of many synnemata also branched laterally and terminally.

F. HOST RANGE OF THE STILBOID FUNGUS

The stilboid fungus was isolated from 29 of sixty locally collected plants, the names of which are listed on pages 118 - 120. Except for isolates nos. 2, 4, 8, and 16, the general appearance, and rate of growth of all 29 isolates was one of similarity when they were subcultured on potato dextrose agar (Plate 43 a and b). Isolate no 2 from Aster simplex produced fewer synnemata than any of the others. Approximately one half of cultures no. 4 showed evidence of mutation and no spores were produced on that half. Zonation was hardly been seen and there was no conidia production at the mutated area. There were a number of whitish mycelia produced superficially on the culture of isolate no. 8 and zonation occurred but was not pronounced. Isolate no. 16 was dark purple in colour with only slightly visible zonation. Most of the isolates had a purplish colour at the center of the colonies where the inoculation took place, and were yellowish at the periphery. In most of the plant specimens, the fungus always appeared first from the root and then the stem of the host. Eventually, it spread to the other parts, including petioles and leaves except in the case of Nicotiana tabacum (isolate no. 12), Lycopersicon esculentum (isolate no. 10), and Solanum melongena (isolate no. 20), where the fungus appeared from the root and the stem separately because they were too large to be put in the same culture dish.

The author failed to isolate the stilboid fungus from bark or wood, places from which many species of Stilbella have been found (Wakefield & Bisby, 1941). The synnemata were never found directly on the living plants, unless the washed root or plant parts were floated on, or partially submerged in water. The synnemata appeared on most of the plants within ten days after they were placed in water but it took 24 days for them to be seen on Solanum melongena, which was the longest time.

Evidence that the fungus occurs in roots, and not merely on their surface, is provided by the fact of its common appearance from roots that had been washed and surfaced sterilized with 10% Javex. The possibility of this fungus being mycorrhizal, as it is suspected of being, needs further investigation.

List Plants Collected in Host Range Experiment

Plants Names	Stilboid Fungus Isolated
<u>Acer saccharum</u> Marsh	-
<u>Achillea millefolium</u> L.	-
<u>Althaea rosea</u> L.	-
<u>Amaranthus retroflexus</u> L.	x
<u>Ambrosia artemisiifolia</u> L.	-
<u>Antirrhinum majus</u> L.	-
<u>Aster simplex</u> Wild.	x
<u>Brassica campestris</u> L.	-
<u>Campanula rapunculoides</u> L.	x
<u>Capsella bursa-pastoris</u> (L.) Medic.	-
<u>Capsicum anuum</u> L.	-
<u>Capsicum frutescens</u> L.	-
<u>Chelidonium majus</u> L.	-
<u>Chenopodium album</u> L.	x
<u>Chenopodium glaucum</u> L.	x
<u>Chrysanthemum leucanthemum</u> L.	x
<u>Cirsium arvense</u> (L.) Scop.	-
<u>Coleus Blumei</u> Benth.	-
<u>Erigeron canadensis</u> L.	-
<u>Erigeron philadelphicus</u> L.	-
<u>Erigeron strigosus</u> Muhl.	x
<u>Helianthus annuus</u> L.	x
<u>Helianthus strumosus</u> L.	-

Plant Names

Stilboid Fungus Isolated

<u>Impatiens sultanii</u> Hook.	x
<u>Iresine Herbstii</u> Hook.	x
<u>Lactuca serriola</u> L.	x
<u>Lepidium densiflorum</u> Schrad.	x
<u>Leontodon autumnalis</u> L.	x
<u>Linaria vulgaris</u> Hill.	-
<u>Lotus corniculatus</u> L.	-
<u>Lycopersicon esculentum</u> Mill	x
<u>Malva neglecta</u> Waltr.	x
<u>Matricaria matricaroides</u> (Less.) Porter	-
<u>Medicago sativa</u> L.	x
<u>Nicotiana tabacum</u> L.	x
<u>Oenothera biennis</u> L.	x
<u>Oxalis stricta</u> L.	-
<u>Pelargonium</u> sp.	x
<u>Phaseolus vulgaris</u> L.	-
<u>Phleum pratense</u> L.	-
<u>Plantago major</u> L.	x
<u>Polygonum convolvulus</u> L.	x
<u>Polygonum persicaria</u> L.	-
<u>Polygonum scabrum</u> Moench.	-
<u>Portulaca oleracea</u> L.	x
<u>Potentilla argentea</u> L.	x
<u>Senecio vulgaris</u> L.	-

Plant Names

Stilboid Fungus Isolated

<u>Setaria glauca</u> (L.) Beauv.	x
<u>Solanum melongena</u> L.	x
<u>Solanum tuberosum</u> L.	-
<u>Solidago canadensis</u> L.	-
<u>Stellaria media</u> L.	-
<u>Taraxacum officinale</u> Weber.	-
<u>Tragopogon pratensis</u> L.	-
<u>Trifolium campestre</u> Schreb.	x
<u>Trifolium hybridum</u> L.	x
<u>Trifolium pratense</u> L.	x
<u>Trifolium repens</u> L.	x
<u>Vicia cracea</u> L.	-
<u>Zea mays</u> L.	-

Plate 43 a and b. The cultures of 21 isolates of the stilboid fungus obtained from the host plants listed below:

Isolate No.	Host Plant
1.	<u>Amaranthus retroflexus</u> L.
2.	<u>Aster simplex</u> Wild.
3.	<u>Campanula rapunculoides</u> L.
4.	<u>Chenopodium album</u> L.
5.	<u>Chrysanthemum leucanthemum</u> L.
6.	<u>Erigeron strigosus</u> Muhl.
7.	<u>Helianthus annuus</u> L.
8.	<u>Impatiens sultani</u> Hook.
9.	<u>Lactuca serriola</u> L.
10.	<u>Lycopersicon esculentum</u> Mill.
11.	<u>Medicago sativa</u> L.
12.	<u>Nicotiana tabacum</u> L.
13.	<u>Oenothera biennis</u> L.
14.	<u>Pelargonium</u> sp.
15.	<u>Plantago major</u> L.
16.	<u>Polygonum convolvulus</u> L.
17.	<u>Portulaca oleracea</u> L.
18.	<u>Potentilla argentea</u> L.
19.	<u>Setaria glauca</u> (L.) Beauv.
20.	<u>Solanum melongena</u> L.
21.	<u>Trifolium repens</u> L.



Plate 43 a.



Plate 43 b.

List of the Stilboid Fungus Isolates which are
not Included in Plate 43 a. and b.

Isolate No.	Host Plant
22.	<u>Chenopodium glaucum</u> L.
23.	<u>Iresine Herbstii</u> Hook.
24.	<u>Lepidium densiflorum</u> Schrad.
25.	<u>Leontodon autumnalis</u> L. *
26.	<u>Malva neglecta</u> Waltr.
27.	<u>Trifolium campestre</u> Schreb.
28.	<u>Trifolium hybridum</u> L.
29.	<u>Trifolium pratense</u> L.

G. HOST PARASITE RELATIONSHIP

1. External Appearance of White Clover Inoculated with the Stilboid Fungus

Plate 44 (a) and (b) show that the white clover plants inoculated with the stilboid fungus by four different methods did not have any evidence of the disease. The plants of all treatments appeared to be externally healthy. Several petioles of the leaves of the control plant (no.1), grew horizontally over the soil in the pot. Plant no. 2 which was inoculated at the leaves, did not show any sign of abnormalities, not even on the leaves where the inoculation was made. It appeared healthy and produced about the same number of leaves as those of the control plant, except that they were all erect instead of being nearly horizontal like those of the control. The plants of treatments no. 3 and no. 5 which were inoculated at the petioles and soil-line respectively, had similar rates and styles of growth. Both produced fewer, but slightly larger leaves than those of no. 1 and no. 2, and neither had any external symptoms of disease. The growth of the plant of treatment no. 4 was retarded because it was retransplanted after root dip inoculation. It had to recover from the shock of transplantation and become adjusted to the soil of a different pot before continuing its growth. Since the plant appeared to be healthy, without visible abnormalities, the growth retardation was probably not due to the effect of the stilboid fungus.

Plate 45 shows representative root systems of plants of each of the treatments. It can be seen that the roots of all the plants are normal and healthy although those of treatments no. 3, and 4 are smaller than the others. Plant no. 4 was expected to have the poorest root system, as explained above. Nodulation occurred, in about the same amount, on all plants except plant no. 4.

The control and inoculated clover plants which were grown in the glass jars, one of which is illustrated in Plate 2, (page 26) all appeared to be healthy. The stilboid fungus was isolated from the tissues of one of the inoculated plants in this treatment. This was done as a check to see if the inoculum had really entered and grown in the plant. In a similar isolation attempt no fungus could be obtained from the control plant. The presence of the stilboid fungus did not appear to have any effect on the growth or external morphology of the host plant in this experiment.

It may be worth noting here that the stilboid fungus grew from some seeds of white clover when they were placed on PDA in a Petri dish. It therefore has the possibility of being a seed borne fungus since the seeds were surfaced sterilized with Javex. This possibility needs to be more thoroughly investigated. The fungus was found in 4 of the 100 seeds tested.

2. Internal Appearance of Plants Infected by the Stilboid Fungus

Plate 46 to 48 illustrate the presence of the stilboid fungus in the root of an inoculated white clover plant grown in a glass jar. Hyphae of the stilboid fungus were limited to the cortex around the vascular system of the roots where they were seen to be intercellular and extracellular (Plate 46 and 47). The presence of the fungus in the cortical tissue did not disturb the plant growth or destroy the tissues. The fungal parasitism appeared to have been very weak because no dead or distorted tissues were observed. A number of hyphae and spores were around some cells, as seen in longitudinal and transverse sections (Plate 48 a. and b.). Apparently, the fungus was inhibited by the endodermis around the vascular system because it was not seen inside these tissues.

The best staining procedure used here was carbol thionin and orange G (Gurr, 1956) for it gave very good differentiation of parasite and host plant tissues. Heidenhain's hematoxylin (Conn et al., 1960), and safranin and fast green (Sass, 1958) also gave fair results. Pianese IIb (Simmons and Shoemaker, 1952) did not give any differentiation of fungus and host tissues; the whole section being stained uniformly pink. In Plates 46 to 48, the sections were stained with carbol thionin - orange G.

The results of this experiment show that the stilboid fungus can be a parasitic fungus. As a parasite it did not

cause visible evidence of disease to the plants in which it lives and grows.

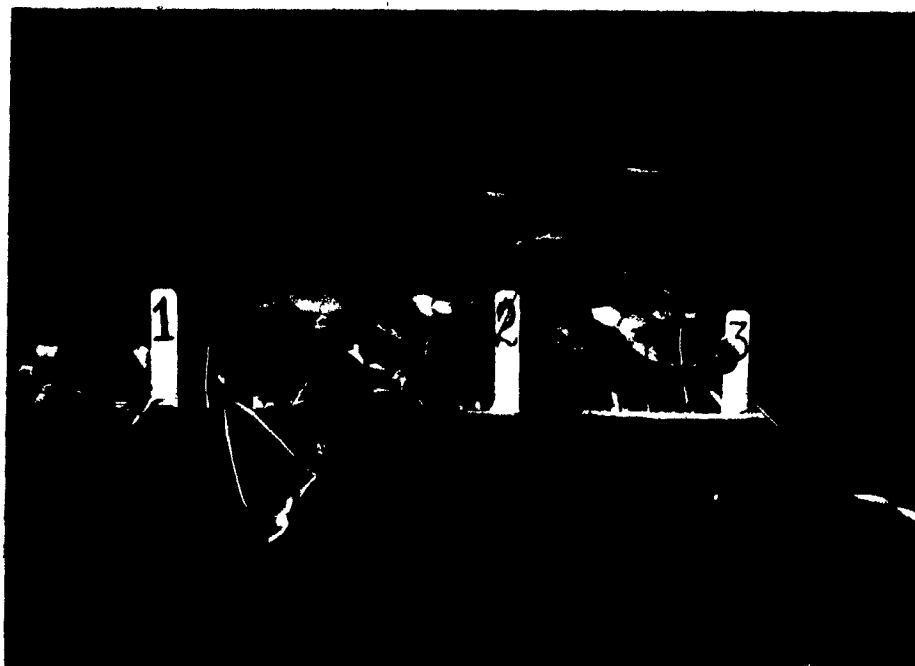


Plate 44 a.



Plate 44 b.

Plate a. and b. Five treatments of two-month old white clover with the stilboid fungus: (1) control; (2) leaves inoculated; (3) petioles inoculated; (4) root dip inoculated; (5) soil inoculated.

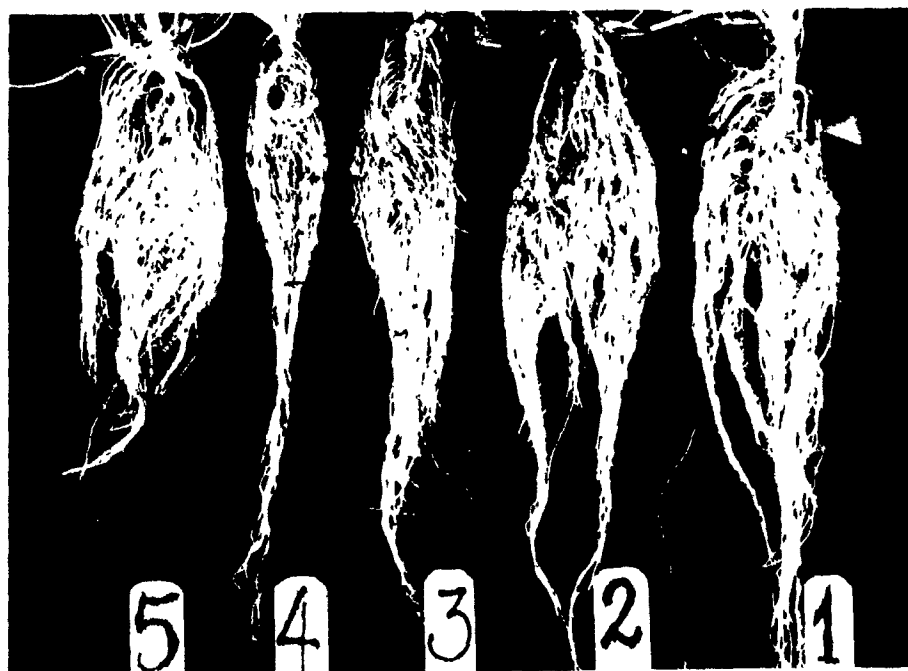


Plate 45. The root systems of white clover plants that had been treated as follow: (1) Control, (2) leaves inoculated with the stilboid fungus; (3) petioles inoculated with the stilboid fungus; (4) root dip inoculated with the stilboid fungus; and (5) soil inoculated with the stilboid fungus.



Plate 46. Longitudinal section of white clover root infected with the stilboid fungus which is shown in the cortex. Mag. 500K.



Plate 47. Transverse section of an infected white clover root showing the stilboid fungus in the cortex intercellularly and intracellularly. Mag. 500X.



Plate 48 (a). Mag. 2000X.



Plate 48 (b). Mag. 4000X.

Plate 48 (a) and (b). Longitudinal (a); and transverse (b) sections of inoculated white clover root showing the stilboid fungus in and between the cells of the cortex.

V. DISCUSSION AND CONCLUSION

The prime objective of this study was to determine whether or not an unnamed stilboid fungus was different from all other previously described species. Emphasis has, therefore, been on the morphological features of the fungus and how these may be modified by certain environmental factors. A survey of the literature had shown that the fungus in question had features similar to Stilbum zacalloxanthum Moore. However, the results of this study show that they are not of the same species. Studies were also made to learn the best temperature, media, and pH for growth and sporulation of the unnamed fungus. Because light is known to affect changes in the life history of many fungi, this too was included in the general study of the fungus. In this instance light had a visual effect on asexual reproduction in that the spore-bearing synnemata of the fungus were produced in rhythms corresponding to the exposures to light. Whether this is an endogenous or exogenous response to light is not certain. The study of light included here was not intended to be extensive. There are many more aspects for further study such as: the effect of different light intensities; the effect of the quality of light, red, blue, green, etc.; the effect of different exposure periods of light of different quality on the development of fruiting structures, etc.; etc.

Finding that morphological abnormalities of the stilboid fungus are induced by volatile metabolites of each of the three species bacteria tested, support the results of many workers that volatile products of some bacteria can cause morphological changes in various fungi. The fact that it was found in surface sterilized clover seeds suggests that it may be seed borne in other host plants. This too, should be worthy of further study by plant pathologists. The stilboid fungus was found to have rather weak competitive ability when grown in culture dishes with other fungi. It is not known to be ecologically or economically important in nature.

The stilboid fungus was found to have a wide host range for it was isolated from many plant species. The nature of the association of this fungus with plant roots is not really understood. A large population in the form of mycelia and conidia was in and between cells of the cortex of the inoculated roots without producing any visible evidence of disease. The physiology of the fungus associated with its host plant tissues is a scope for future research to understand this relationship. At present, based on the results of this study, indications are that this stilboid fungus is a symptomless, systemic parasite of many plants.

VI. SUMMARY

An unnamed stilboid fungus is described which is now assumed to be a new species of the genus Stilbella Lindau, family Stilbellaceae, class Deuteromycetes. It was first isolated from red clover (Estey, 1960) but has now been isolated from white clover and from other species of plants when they were floated or partially submerged in water. It was also found associated with decomposing plant materials or plant debris. It did not cause any visible disease of the plants used in this study.

The morphology of this stilboid fungus is described. The hyphae are narrow (1.6 micrometers wide); hyaline and septate. The conidiophores are simple or branched and undistinguished. There are two kinds of conidia produced; macroconidia and microconidia. The sizes of macroconidia are 1.5-6.4 (width) x 11.5-18.0 (length) micrometers and those of microconidia are 1.5-5.7 (width) x 3.5-9.5 (length) micrometers. They are borne singly as blown out ends at the tip of the conidiophores. They germinate by means of germ tubes. The hyphae aggregate into an erect column to form a bright yellowish synnema. The conidiophores arise from the apex of the aggregated hyphae and produce a number of conidia which

then adhere together to form a slimy headed synnema. The synnemata are up to 2 mm high; and the widths of the columns are up to 45 micrometers. The morphology of Stilbum zacalloxanthum Moore was also studied by the author to compare with the unnamed stilboid fungus. The result is that they were different in shape and size and colour.

The physiological study found that the unnamed stilboid fungus had maximum growth and sporulation at 25°, on czapek solution agar, and at pH 5.6 under the experimental conditions. The linear mycelial growth reached 26 millimeters after ten days. Zonation was induced by alternating periods of light and dark and on czapek solution agar. Light also effected the increased growth rate and sporulation. The same study on light for Stilbum zacalloxanthum Moore found that light also influenced its zonation but retarded its growth and sporulation.

The unnamed stilboid fungus was weak when grown to compete with the twenty fungi tested. The volatile products of the three species of bacteria tested induced morphological abnormalities in the hyphae. The fungus has a wide host range. Twenty-nine isolates were obtained from the 60 plant species collected from different locations. The fungus was also found arising from surface sterilized clover seeds. It was present inter and intra-cellularly in the tissues of the

cortex of the roots of inoculated white clover plants without causing any death or distortion of tissues and no external symptoms. The host plants were normal without any sign of disease.

VII. REFERENCES

- Ainworth, G.C. and G.R. Bisby. 1954. A dictionary of the fungi. 2nd. ed. The Commonwealth Mycological Institute, Kew, Surrey, England. 475 pp.
- Alexopoulos, C.J. 1962. Introductory mycology. 2nd.ed., J. Wiley & Sons, New York. 613 pp.
- Anon, 1968. Plant pathologist's pocket book. Commonwealth Mycological Institute, Kew, Surrey, England. 267 pp.
- Aytoun, R.S.C. 1956. The effects of griseofulvin on certain phytopathogenic fungi. Ann. Bot. (London) n.s. 20: 297-305.
- Barathova, H., V. Betina, and P. Nemec. 1969. Morphological changes induced in fungi by antibiotics. Folia Microbial (Prague) 14:475-483.
- Barnett, H.L. 1960. Illustrated genera of imperfecti fungi. Burgess Publishing Co., Minneapolis. 218 pp.
- Barnett, H.L., and V.G. Lily. 1966. Manganese requirements and deficiency symptoms of some fungi. Mycologia 58:585-591.
- Barron, G.L. 1968. The genera of Hyphomycetes from soil. The Williams & Wilkins Co., Baltimore. 364 pp.
- Benjamin, C.R. 1968. Typification of the family Stilbellaceae Taxon 17: 521-527.
- Bessey, E.A. 1950. Morphology and taxonomy of fungi. Blakiston Co., Philadelphia. 336 pp.
- Bitting, K.G. 1920. The effect of certain agents on the development of some moulds. National Capital Press Inc., Washington. 177 pp.
- Callen, E.O. 1940. The morphology, cytology, and sexuality of the homothallic Rhizopus sexualis (Smith) Callen. Ann. Bot. (London) n.s. 4: 791-818.
- Carlile, M.J. 1965. The photobiology of fungi. Ann. Rev. Pl. Physiol. 16: 175-202.
- Childs, E.A., J.C. Ayres, and P.E. Koehler. 1971. Differentiation in Aspergillus flavus as influenced by L-canavanine. Mycologia 63: 181-184.

- Clements, F.E., and C.L. Shear. 1931. The genera of fungi. H.W. Wilson Co., New York. 496 pp.
- Cochrane, 1958. Physiology of fungi. John Wiley & Sons, Inc. New York. 524 pp.
- Conn, H.J., M.A. Darrow, and V.M. Emmel. 1960. Staining Procedures. The Williams & Wilkins Co., Baltimore. 289 pp.
- Donk, M.A. 1963. The generic names proposed for Hymenomycetes XIII. Taxon 12: 153-168. (Stilbum, P. 166).
- Duckworth, R.B., and G.C. Harris. 1949. The morphology of Penicillium chrysogenum in submerged fermentation. Trans. Brit. Mycol. Soc. 32: 224-235.
- Elarosi, H. 1971. Fungal associations. II. Cultural studies on Rhizoctonia solani Kuhn, Fusarium solani Snyder and Hansen, and other fungi and their interactions. Ann. Bot. (London) n.s. 21: 569-585.
- Estey, R.H. 1960. A stilboid fungus from the rhizosphere of red clover. Proc. Can. Phytopath. Soc. 27:13 (Abstr.).
- Fergus, C.L. 1964. Thermophilic and thermotolerant molds and Actinomycetes of mushroom compost during peak heating. Mycologia 56: 267-284.
- Fries, N. 1973. Effects of volatile organic compounds on the growth and development of fungi. Trans. Brit. Mycol. Soc. 60(1): 1-21.
- Gilman, J.C. 1945. A manual of soil fungi. Collegiate Press. Ames, Iowa. 392 pp.
- Gilman, J.C. 1957. A manual of soil fungi. 2nd.ed. Iowa State Coll. Press. Ames, Iowa. 450 pp.
- Gurr, E. 1956. A practical manual of medical and biological staining techniques. London: Leonard Hill (Books) Ltd. 451 pp.
- Hughes, S.J. 1953. Conidiophores, conidia and classification. Can. J. Bot. 31: 577-659.
- _____. 1958. Revisiones Hyphomycetum aliquotcum appendice de nominibus rejiciendis. Can. J. Bot. 36:727-836.

- Hughes, S.J. 1959. Starting point for nomenclature of Hyphomycetes. *Taxon* 8: 96-103.
- Kendrick, W.B., and J.W. Carmichael. 1973. Hyphomycetes. p. 323-503, in "The Fungi" Vol. IV A. G.C. Ainsworth, F.K. Sparrow and A.S. Sussman. (edit.). Academic Press, New York. 621 pp.
- Lanjouw, J. chmn. 1961. International code of botanical nomenclature. *Internat. Bur. Plant. Tax. Nomencl.*, Utrecht. 372 pp.
- Links, J., J.E. Rombouts, and P. Keulen. 1957. The "bulging factor", a fungistatic antibiotic produced by a Streptomyces strain, with evidence of an active water-excreting mechanism in fungi. *J. Gen. Microbiol.* 17: 596-601.
- Mains, E.B. 1948. Entomogenous fungi. *Mycologia* 40: 402-416.
- March, P.B., E.E. Taylor, and L.M. Bassler. 1959. A guide to the literature on certain effects of light on fungi, reproduction, morphology, pigmentation, and, phototropic phenomena. *Plant Disease Reprtr. Suppl.* 261: 251-312.
- Martin, G.W. 1961. Key to the families of fungi. p. 497-519, in "The Fungi", G.C. Ainsworth & Bisby's dictionary of the fungi. 5th ed. Commonwealth Mycological Institute, Kew, Surrey, England.
- McQuade, A.B. 1963. Morphogenesis and nutrition in the Menionella-Stachybotrys group of fungi. *J. Gen. Microbiol.* 30: 429-435.
- Mason, E.W., and M. B. Ellis. 1953. British species of Periconia. *Commonw. Myc. Inst., Myc. Papers*, No.56, 127 pp.
- Moore, R.T., J.H. McAlear, and G.B. Chapman. 1959. Stilbum zacalloxanthum: Taxonomy, and conidial structures as seen by electron microscopy of thin sections. *The Amer. Naturalist*.63(868): 41-43.
- Moore-Landecker, E., and G. Stotzky. 1973. Morphological abnormalities of fungi induced by volatile microbial metabolites. *Mycologia* 65(3): 519-530.
- Morris, E.F. 1963. The synnematus genera of the fungi imperfecti. *Biol. Sci. No. 3*, Western Illinois University, Macomb, Ill. 143 pp.

- Napier, E.J., D.I. Turner, and A. Rodes. 1950. The in vitro action of griseofulvin against pathogenic fungi of plants. *Ann. Bot. (London)* n.s. 20: 461-466.
- Page, R.M. 1965. The physical environment for fungal growth. 3. Light. p. 599-571, in "The Fungi an Advanced Treatise". Vol I. G.C. Ainsworth & A.S. Sussman. (edit.) Academic Press, New York. 748 pp.
- Park, D., and P.M. Robinson. 1964. Isolation and bioassay of a fungal morphogen. *Nature* 203: 988-989.
- Petch, T. 1945. Stilbum tomentosum Schrad. *Trans. Brit. Myc. Soc.* 28: 101-108
- Sansome, E. 1946. Induction of "gigas" forms of Penicillium notatum by treatment with camphor vapour. *Nature* 157: 843-844.
- Sass, J.E. 1958. Botanical microtechnique. Iowa State College, Ames, Iowa. 228 pp.
- Simmons, S.A., and R.A. Shoemaker. 1952. Differential staining of fungus and host cell using a modification of Pianese IIIB. *Stain. Tech.* 27: 121.
- Smith, A.L. 1903. Notes on a species of Stilbum. *Trans. Brit. Myc. Soc.* 2: 25-26.
- Strzelczyk, A.B. 1968. Influence of antifungal vapors on spore germination of fungi isolated from deteriorated old books. *Can. J. Microbiol.* 14: 901-906.
- Sutton, B.C. 1973. Hyphomycetes from Manitoba and Saskatchewan, Canada. *Commonw. Myc. Inst., Myc. Papers*, No. 132: 143 pp.
- Tubaki, K. 1966. Sporulating structures in fungi imperfecti. page 113-131; in "The Fungi an Advanced Treatise" Vol. II; by G.C. Ainsworth & S.S. Sussman (edit.). Academic Press, New York. 805 pp.
- Verdcourt, B. 1952. The effect of phenolic compounds on the germination and growth of microfungi. *Mycologia* 44: 377-386.
- Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159: 850.
- Wakefield, E.M., and G.R. Bisby. 1941. List of Hyphomycetes recorded for Britain. *Trans. Brit. Myc. Soc.* 25: 49-126.

Zalokar, R.S. 1962. A preliminary study of the genus
· Stilbella. Western Illinois University. 100 p.
- (Unpublished Master's thesis).