BIOLOGICAL STUDIES OF *LILLIPUTIA MARGARITACEA* (N. S.)

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Leila Jean Saunders Walker

1950

This is to certify that the

thesis entitled

BIOLOGICAL STUDIES OF
LILLIPUTIA MARGARITACEA (N.S.)

presented by

LEILA JEAN SAUNDERS WALKER

has been accepted towards fulfillment
of the requirements for

M.S. degree in BOTANY

Major professor

Date DECEMBER 5, 1950

O-169

BIOLOGICAL STUDIES OF *LILLIPUTIA ALBO-RITICEA* (N.S.)

by

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A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1950

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ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. C. J. Alexopoulos for his valuable time, approval of the thesis problem, the photographs used in PLATES IV and V, for his guidance, patience, and criticisms; Dr. Ernst Bessey for the original cultures, suggestion of the problem and many translations; Mr. W. H. Seaman of the Department of Foreign Language for the translation of the technical description into Latin; Mrs. Marie Moour for making available many materials necessary to complete this work; and members of the faculty and staff of the Botany and Bacteriology Departments whose cooperation made this thesis possible.

I especially want to thank Dr. Ray Nelson for instruction in photographic work, use of his equipment, and for valuable years of training in laboratory methods.

L. J. S. W.

I. INTRODUCTION

A fungus, growing on stable manure was collected by Dr. Ernst Bessey, at East Lansing, in the fall of 1944. Upon examination, pearl-like structures and small glistening hyaline mucous droplets were found on the substrate.

In order to determine the taxonomic position of the fungus, it was necessary to isolate and germinate single cells, and to study the behavior of this fungus under different conditions of nutrition, and environmental factors. These studies constitute the body of this thesis.

The germination of a single cell (conidium or ascospore) resulted in the production of the asexual stage belonging to the form genus Gliocladium, and the perfect stage which proved to be a species of Lilliputia.

As will be seen from the body of this thesis, the results of the present study indicate that the fungus under consideration is a new species. At the suggestion of Dr. Bessey the name Lilliputia margaritacea is hereby proposed for this fungus in accordance with the following description:

Lilliputia margaritacea, sp. nov.

Mycelium hyalinum, laeve. Conidiophorae ~~rare~~ ramosae, symmetricae, plerumque 411 x 20μ (280-431 x 9.4-21μ), 2 - 4 ramis 4.3 - 5.5 x 25.8 - 34.4μ, singulis habentibus 3 - 4 metulas 3.4 - 4.3 x 21.5μ, quae singulae 2 phialides 2.2 - 3.4 x 25.8 x 43μ ferunt. Tria genera conidiorum hyalinorum, laevium, unicellularium generantur: Conidia ellipsoidea 1.6 - 2.4 x 4.4 - 10μ, ovalia 3.4 - 3.8 x 6.7 - 7.8μ et sphaerica 6 - 9μ ordine in phialibus generata et gutta mucosa cohaerentia. Perithecia 400 - 700μ, plerumque 500μ, per spiram myceli generata; cerosa, capillis papillatis;

pariete eorum laxo et duro primo, concreto et molli in maturitate. Asci hyalini spinas ~~habentibus quae longitudinem medi~~ diametri sporae in asco sedentis habent. Ascosporae maturae 19.3 - 21.5μ spinosae, ferrugineo-brunneae. Spinae 1.9 - 2.8μ longae.

Ab E. A. Bessey collecta in East Lansing, Michigan autumno anni 1944 ex sterco.

(English description)

Mycelium hyaline, smooth. Conidiophores sparingly branched, symmetrical, average 411 x 20μ with a range of 230 - 431 x 9.4 - 21μ, two to four branches 4.3 - 5.5 x 25.8 - 34.4μ each having three to four metulae 3.4 - 4.3 x 21.5μ which in turn bear two phialides each 2.2 - 3.4 x 25.8 - 43μ. Three types of hyaline, smooth, unicellular conidia are produced; ellipsoidal 1.6 - 2.4 x 4.4 - 10μ, ovoid 3.4 - 3.8 x 6.7 - 7.8μ and spherical 6 - 9μ, conidia produced successionally upon phialides and held together by a mucilaginous droplet. Perithecia 400 - 700μ, mostly 500μ, produced by the coiling of mycelium; waxy, with papillate hairs; wall loose and hard at first, compact and soft at maturity. Asci hyaline 24.4 - 30 x 26.5 - 55.9μ, subglobose, bulging with 8 aculeate hyaline ascospores having spines $\frac{1}{2}$ diameter of the spore in length while still in the ascus. Mature ascospores 19.3 - 21.5μ echinulate and rust brown in color. Spines 1.9 - 2.8μ long.

Collected by E. A. Bessey in East Lansing in the fall of 1944 on stable manure.

II. REVIEW OF LITERATURE

Lilliputia Boudier et Patouillard (5) is described as somewhat resembling Terfezia in so far as the spores are concerned. The perithecia are described as having a cortical portion which is very thick and much differentiated around the gleba. A great number of asci are found in the jelly-like matrix. The spores are yellow-brown inside a very white perithecium.

In 1887 Winter suggested a possible relationship between Gliocladium penicillioides Corda (3) and Eurotium insigne (26). He mentioned conidia but gave no characteristics for them. Later Petch concluded that this was not the imperfect stage for E. insigne but named G. macropodinum in its place.

In 1910 Bainier (2) described Gliocladium prolificum. A perfect stage was found which was thought to belong to the Perisporaceae.

In 1946 Dennis and Wakefield (9) suggested that Eurotium insigne Winter, Lilliputia gaillardi Boud. et Pat., and G. penicillioides Corda, are the same species and the new combination Lilliputia insignis was proposed for this fungus and the other three names are synonymous. This was placed into Eurotiaceae near Eurotium.

Eurotium was first suggested as the perfect stage of the genus Gliocladium. In 1946 Dennis and Wakefield named Lilliputia as the perfect stage. The above includes a review of those species of Gliocladium having a sexual stage resembling that of our fungus, and those associated with the two species of Lilliputia hitherto described.

III. MATERIALS AND GENERAL METHODS

A. Cultures

Of the twelve original cultures made by Dr. Bessey in 1944, only one, #11, was revived and used for further study. Others were either dead or contaminated. Manure extract was used in an attempt to revive these cultures. The medium was prepared in accordance with the following directions:

Fill one half of flask with horse manure.

Add water to the top of flask.

Steam and stir frequently for one hour in an Arnold Steamer.

Filter through several thicknesses of cheese cloth.

Sterilize at 15 pounds pressure for 1½ hours.

This extract can be stored for further use.

2% Manure Extract Agar

2cc. Manure extract
2 gms. agar
98cc. distilled water

Cultures were examined at intervals in mounts of lacto-phenol, or lacto phenol and cotton blue which stained the fungus light blue showing the phialides well.

All measurements were made in Lacto phenol mounts with a Filar Micrometer.

To prevent contamination Petri plates containing fungus cultures which were kept in incubators for 10 days, were sealed with scotch tape. Previous experimentation showed no effects on growth due to a possible decrease in oxygen.

III B. Media used

Media used throughout this work are listed as follows:

Coons synthetic agar

7.2 gms. saccharose
5.6 " dextrose
1.23 " $MgSO_4$
2.72 " KH_2PO_4
2.02 " KNO_3
1.5% agar
1000 cc. distilled water

Westergaard and Mitchell's synthetic medium

.1 gm. KNO_3
.1 gm. KH_2PO_4
.05 " $MgSO_4$
.01 " $CaCl_3$
.01 " NaCl
5ug per liter biotin
2 gms. sucrose
1.5% agar
.1cc. Beadles' trace elements (pg. 15)
100 cc. distilled water. Adjust to pH 6.0

Richard's synthetic medium

1.0 gm. KNO_3
.5 " $KHPO_4$
.25 " $MgSO_4$
Trace $FeCl_3$
3.43 gms. Saccharose
100 cc. distilled water

Fries synthetic (less tartrate) Mitchell's modification of Fries[1] so as to remove the tartrate.

4.0 gms. Ammonium nitrate
4.0 gms. KH_2PO_4
.2 " NaCl
.2 " $CaCl_2$
1.0 " $MgSO_4.7H_2O$
1000cc. distilled water
adjust to Ph 6.0

---

[1]Original Fries formula (double strength) (3) as follows:

2.0 gms. Ammonium nitrate
10.0 gms. Ammonium tartrate
2.0 gms. KH_2PO_4
.2 gms. NaCl
.2 gms. $CaCl_2$ (anhydrous)
1.0 gms. $MgSO_4.7H_2O$
1 liter water
adjust to pH 6.0
(Sucrose, biotin and trace elements not added)

Czapek's synthetic agar

.5 gm. $MgSO_4$
1.0 " K_2HPO_4
.5 " KCl
2.0 " $NaNO_3$
3-4 " saccharose
1000cc. distilled water
1.5% agar

Corn meal beer agar

100cc. beer
20 gms. Difco corn meal agar
2 " sucrose
water to 1000cc.

Wheat agar

100 gms. wheat
1000cc. distilled water
1.5% agar
Infuse wheat for one hour at 50°C. (wrap in cheese cloth and suspend in water).
Decant, add agar, steam for 20 minutes.
When clarity is necessary, filter through glass wool.
Sterilize at 12 lbs. pressure for 20 minutes.

Bacto beef heart infusion

This is a liquid infusion medium for the cultivation of fastidious organisms.

Oat agar

100 gms. ground oats
1000cc. distilled water
Infusion for one hour as in wheat agar
1.5% agar

Potato dextrose agar

200 gms. peeled and sliced potatoes
1000cc. distilled water
Steam for one hour, decant.
1.5% agar
15 gms. dextrose

Bacto bean agar

22.5 gms. Bacto bean agar
1000cc. distilled water
Sterilize at 15 lbs. pressure for 20 minutes.

Rice agar

100 gms. polished rice
1000cc. distilled water
Infuse for one hour as in wheat agar
1.5% agar

Red clover stems

Four 2 inch long stems placed in test tubes.
5cc. water (distilled) in each tube.
Steam sterilize (one hour each day for 3 successive days.)

Carrot plugs

Cut carrots to fit test tubes in slant-like strips.
Add few cc. distilled water
Steam for one hour for 3 successive days.

III, C. Nitrogen and Carbon Equivalents

The basic medium (Coons synthetic agar) was sugar free.

Two constant sources of nitrogen used were potassium nitrate and a-asparagine. The 2.029 grams of KNO_3 used per liter of Coons synthetic medium contain .280 gms. of nitrogen. Asparagine having the same equivalent was used (1.320 grams of a-asparagine contain .280 grams of nitrogen per liter.)

With these two constant nitrogen sources, various molarities of sugars were used. Two sets of each molarity of sugar were prepared in 50cc. aliquots and each set run in triplicate. KNO_3 was used as a nitrogen source in one set and a-asparagine in the other (TABLE 1).

TABLE 1. AMOUNTS OF SUGAR AND CARBON EQUIVALENTS IN MEDIA EMPLOYED; NITROGEN CONSTANT.

Sugar	Molar Conc.	Flask No.	Gms. per Flask (50 ml. aliquot)	Gms. per liter	Gms. C per liter
Dextrose M. W.[1] 198.17	.03	1	.297	5.95	2.15
	.06	2	.594	11.39	4.33
	.09	3	.892	17.84	6.43
	.12	4	1.189	23.79	8.63
	.15	5	1.486	29.74	10.79
Saccharose M. W. 342.2	.03	1	.510	10.27	4.32
	.06	2	1.030	20.53	8.64
	.09	3	1.540	30.80	12.96
	.12	4	2.050	41.06	17.27
	.15	5	2.560	51.32	21.59
Lactose M. W. 342.2	.03	1	.510	10.27	4.32
	.06	2	1.030	20.53	8.64
	.09	3	1.540	30.80	12.96
	.12	4	2.050	41.06	17.27
	.15	5	2.560	51.32	21.59
Galactose M. W. 180.15	.03	1	.270	5.40	2.15
	.06	2	.540	10.80	4.33
	.09	3	.810	16.21	6.48
	.12	4	1.080	21.61	8.63
	.15	5	1.350	27.01	10.79
Maltose M. W. 342.2	.03	1	.510	10.27	4.32
	.06	2	1.030	20.53	8.64
	.09	3	1.540	30.80	12.96
	.12	4	2.050	41.06	17.27
	.15	5	2.560	51.32	21.59

[1] Molecular weight

IV. EXPERIMENTAL WORK

A. Relationship of the Lilliputia and Gliocladium Stages

1. Single cell isolations

a. Germination of conidia

A duplicate series of water blanks containing various amounts of water, at .1cc. intervals from .1cc to 1cc, was prepared. One pair of water blanks for each interval was inoculated with conidia obtained by applying a sterile platinum wire to a single conidial head. Each tube was shaken to separate the individual spores so that when the contents was poured onto a plate containing solidified 2% manure agar and the plate rotated, the spores spread fairly evenly on the surface. Each plate of filtered 2% manure agar contained about 15cc. of agar. The plates were inverted on the stage of the microscope and observed through the low power objective. As spores were found, an India ink mark was made on the glass so that the spores could be located thereafter. Best results were obtained with the .5cc water blanks. The spores were spread out sufficiently so that single germinating spores could be cut out readily and transferred to 2% agar plates. The majority of conidia had germinated 13 hours after inoculation. Hyphal tips were cut from the resulting colonies and transferred to test tubes containing 2% manure agar. All single cell isolations and hyphal tip inoculations produced the Gliocladium stage within a few days, and the perfect stage within 10 days (Plate 1, Figure 1).

Iv. A, I, b. Germination of ascospores

The same method used in germinating conidia was also used in determining the best dilution for ascospore isolations. In this case, however, a single mature perithecium (the surface of which sometimes

bore conidia) was transferred to each sterile water blank and crushed with a sterile glass rod. Each dilution was shaken for 60 seconds to separate the ascospores, poured on a cooled 2% filtered[1] manure agar plate. The plates were rotated, and after the excess moisture had evaporated, they were inverted on the stage on the microscope and marked with India ink to indicate positions of the ascospores. Best results were obtained with one crushed perithecium in .5cc. of sterile tap water. These plates were examined daily until the agar had almost dehydrated but no germinated spores were found. This procedure was repeated three times with the same results.

Heat was used in an attempt to germinate ascospores. Four sterile test tubes containing ascospores suspended in .5cc. sterile distilled water were prepared, and placed in water baths at the following temperatures indicated below, and poured onto 15cc. 2% manure agar.

Tube #1-- 70°C for 10 minutes.
Tube #2-- 90°C for 5 minutes.
Tube #3--60°C for 10 minutes.
Tube #4-- 50°C for 10 minutes.

No germinations had occurred up to the 10th day.

Hydrochloric acid was chosen in a third attempt to germinate the ascospores. Since the fungus had been found on manure, the following experiment was set up. According to Dukes (10), the HCl range of the stomach of the horse lies between .14 and.21%. The statement that the stomach emptied its contents completely within two and one half days was taken into consideration. The following dilutions of HCl were prepared: .14%, .15%, .16%, .17%, .18%, .19%, .20%, and .21%. Two tenths of a ml.

---

[1]For all single spore isolations, media were filtered through washed glass wool.

of each dilution was transferred to a test tube, sterilized, and inoculated with one mature perithecium which later was crushed with sterile rod. Three sets of dilutions were prepared; one set "digested" one day, another two days and the last, two and one half days.

Out of five repetitions, germinations occurred only twice at .16 and 21% (PLATE 1, Figure 2). Since HCl treatment killed all conidia which were transferred to the water blanks with the perithecia, the resulting germinating ascospores were known to be "pure". For this reason all single cell germinations derived from HCl treatment were used or stock cultures throughout all experiments.

Ascospores were induced to germinate by freezing in a refrigerator freezing compartment at -6°C for 18 hours. A perithecium was crushed in a sterile test tube containing .5cc. of sterile tap water and placed in the -6°C freezing compartment. After 18 hours, the tube was allowed to thaw and the contents then poured onto a cooled 2% manure agar plate and rotated. Germination of ascospores and conidia occurred.

Colonies from all single cell isolations (ascospore or conidium) produced both perfect and imperfect stages.

IV, B. Effect of Various Factors on Growth and Reproduction

1. Nutrition

a. Organic media

In an attempt to produce both stages of the fungus in abundance and in a comparatively short time, the following media were inoculated: oat, manure (concentrated), 5% manure, rice, wheat, potato dextrose, corn meal, beer and 2% agars. Liquid wheat medium and clover stems sterilized by tyndallization were also used. Triplicate plates were made of each medium and inoculated with a perithecium. Growth measurements were made each day for 10 days and all plates were kept at 26°C.

The resulting growth measurements and microscopic observations are in TABLE II and III.

Generally organic media produced comparatively thick growth as well as both stages of the fungus in a short time.

In wheat agar, conidia and perithecia were found in abundance (about 800 perithecia per plate).

Rice medium produced as many perithecia but the conidial stage was not as abundant.

The only explanation the author can give for the absence of growth in concentrated manure agar is that a new batch of manure extract had been prepared since the initial experiments and the manure may have been too old and toxic.

TABLE II. Growth on Various Organic Media as Represented by Colony Diameters in mm.* (10 Days' Growth at 26°C)

Media	Days									
	1	2	3	4	5	6	7	8	9	10
Rice agar	0	7.6	13.6	18	20	26.6	29.3	33	42.3	50.0
Oat agar	0	11.3	24.3	34	43.3	51.3	52.6	55	66	77
Wheat agar	0	6.6	11.6	16	22	30	39.3	50	55	60
Manure agar (concentrated)	0	0	0	0	0	0	0	0	0	0
Manure agar 3%	0	8	12	16	20.6	24	28	36	43.6	48
Potato dextrose agar	0	5.6	3.3	12	17.6	24	26	30	34.3	38.6
Water 2%/agar	0	5.6	7.5	9.3	12.6	13	16.6	13	20.3	22.6
Corn meal beer agar	0	0	0	0	0	0	0	0	0	0
Liquid wheat medium	On the tenth day a pellicle formed over three fourths of the surface of 15cc. in a 50 cc. flask.									
Clover stems	0	0	0	0	0	0	0	0	0	0

*Figures represent the mean diameter of three plates.

TABLE III. Growth on Various Organic Media as Represented by Macroscopic Observations. (10 days' growth at 26°C).

Media	Perithecial production	Conidial production	Mycelium
Rice agar	Abundant; 7 days after inoc.	Abundant	Dense growth
Oat agar	Very few; 7 days after inoc.	None	Dense growth; atypical
Wheat agar	Abundant; 5 days after inoc.	Abundant; 4 days after inoc.	Very dense
Manure agar (conc)	None	None	None
Manure agar 3%	Sparse; 8 days after inoc.	Sparse; 3 days after inoc.	Sparse
Potato Dextrose agar	None	Abundant; 6 days after inoc.	Very dense
water 2%/agar	None	None	None
Corn-meal Beer agar	None	None	Dense
Clover stems	None	None	None
Liquid wheat medium	None	None	Pellicle over 3/4 surface of medium

IV, B, 1, b. Synthetic Media

Growth of Lilliputia margaritacea on Coons, Czapek's, Fries, Richard's and Westergaard and Mitchell's synthetic media is shown in TABLES IV and V.

Fries modified synthetic medium was prepared and Beadles trace elements were added in the amount of 1ml. per liter.

Beadles Trace elements

Bo- .01 mg. per l
Co- .1 " "
Fe- .2 " "
Mn- .02 " "
Mo- .02 " "
Zn-2.0 " "

Fries modified synthetic medium (see Materials and Methods) was prepared in double strength (Fries 2X) and the following combinations were made:

(A) 250cc. Fries 2X
20 gms. sucrose
(STOCK SOLUTION)

(B) 50cc. (A)
2 gms. malt extract
.5 gms. yeast extract
Enough water to make 100cc.

(C) 50cc. (A)
.5 gm. yeast extract
Enough water to make 100cc.

(D) 50cc. (A)
.4cc. biotin (prepared as 5γ per cc. in 50% alcohol.)
4cc. vitamin mixture (see below)
Enough water to make 100cc.

Vitamin mixture

B_1	25 mgs.	Inositol	100.0 mgs.
B_2	12.5 "	Pimelic	100.0 "
B_6	12.5 "	Choline	25 "
Pantothenic acid	200 "	Hyd. yeast (N.A.)	250 "
Para amino benzoic acid	12.5 "	Folic acid	.05 "
Nicotinamide	50 "	Water	250cc.

(E) 50 cc. (A)
1 gm. succinic acid
.4cc biotin
4cc. vitamin mixture
Enough water to make 100cc.

(F) 50cc. (A)
Enough water to make 100cc.
(CONTROL)

(G) 50cc. wheat agar
(CONTROL)

All media were adjusted to pH 6.0

Two 15cc. aliquots of each of the above media (B, C, D, E, F, and G) were prepared, 15cc. agar added, and the sterilized media inoculated with a perithecium.

Medium B- Growth; sparse, no fruiting

Medium C- Growth; abundant. Perithecia and conidia abundant.

Medium D- Growth; trace, no fruiting

Medium E- No growth

Medium F- No growth

Medium G- Growth; sparse as compared to C. Fruiting; fair amount.

Westergaard and Mitchell (38) describe a possible synthetic medium for abundant formation of the sexual stage of Neurospora.

100cc. of this medium were prepared. To one half of this quantity .5% yeast extract was added, and the rest was used as a control containing no supplement.

The resulting growth was compared to that in Fries modified synthetic medium (plus .5% yeast extract). Westergaard and Mitchell's synthetic medium plus .5% yeast extract allowed the production of more perithecia than did Fries, but maturation of the perithecia was prolonged. Growth in Westergaard and Mitchell's medium without supplement was the same as that in Fries modified synthetic without supplements.

TABLE IV. Ten Days Growth of Lilliputia margaritacea at 26°C on Various Synthetic Media as Represented by Average Diameter of 3 Plates in mm.*

Media	Days									
	1	2	3	4	5	6	7	8	9	10
Coons Agar	0	2	2.6	2.6	3.6	4.0	4.5	5.0	6.5	7.8
Czapek's Agar	0	8	13.5	15	25	26	31	33	44	47
Richard's agar	0	1	1.5	2	3.5	4.0	5.5	7	7	7
Fries Modified plus .5% yeast extract	0	5	10	13	22	30	35	41	43	55
Westergaard and Mitchell's Agar	0	2.5	5	10	16	20	22	24	26	28

*
All measurements recorded in mm., representing mean diameter of three plates.

TABLE V. Fruiting of Lilliputia margaritacea on Various Synthetic Media as Represented by Macroscopic Observations (10 days' growth at 26°C).

Media	Perithecial production	Conidial production	Mycelium
Coons agar	none	none	sparse
Czapek's agar	none	abundant	thin
Richard's agar	none	none	dense
Fries mod. agar plus .5% yeast extract	Abundant; 4 days after inoc.	Abundant; 6 days after inoc.	dense
Westergaard & Mitchell's agar	Abundant; 7 days after inoc.	sparse	dense

IV, B, 1, b, 1). Carbon metabolism

A modification of Coons synthetic medium was used as a base for the study of carbon metabolism of this fungus. Various molarities (.03, .06, .09, .12, .15 molar) of each of five sugars were used as carbon sources in the various experiments conducted. These were combined with an organic and inorganic source of nitrogen (a-asparagine and KNO_3). The amount of KNO_3 and a-asparagine used, contained .230 gms. of nitrogen per liter.

All plates were run in triplicate and each plate was inoculated with a perithecium. Measurements of colony growth were made each day for 10 days.

The results of these experiments are summarized in TABLES VI to X and GRAPHS 1 and 2.

IV, B, 1, b, 2) Nitrogen sources

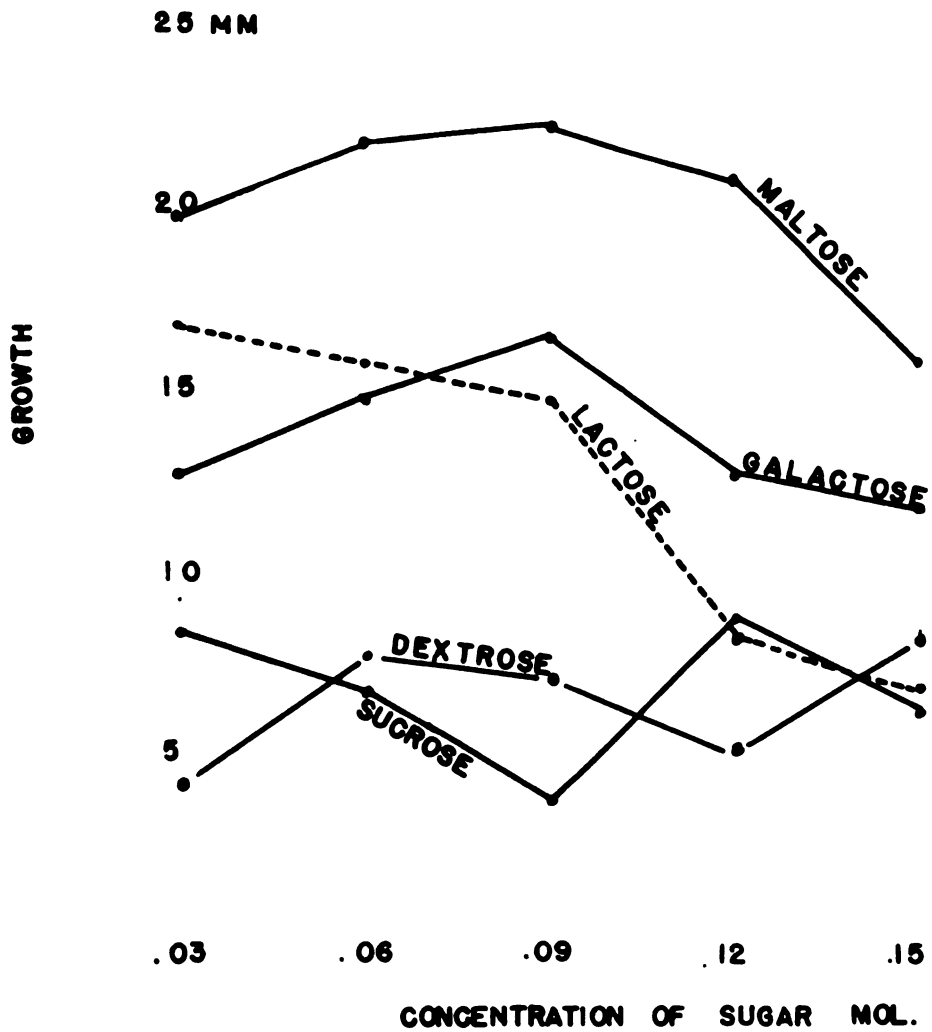
A modified Coons synthetic medium was used as a base for the study of nitrogen metabolism of Lilliputia ranunculacea. The following amounts of urea were used as nitrogen sources in these experiments; .01, .1, .3, and .6 gms. per liter. Plates were run in triplicate, inoculated, and incubated for 13 days.

Maltose	3.03	gms. per 100cc.	Results:
$MgSO_4$	.123	" " "	.60 gms. urea per
Urea	.01 to .6	gms. per 1000cc.	liter produced
KH_2PO_4	.272	gms. per 100 cc.	largest growth
Agar	1.5%		(72mm); mycelium
Distilled water	100cc.		sparse.
Trace Fe			

Ammonium acid phosphate

The following medium was prepared in triplicate, inoculated with a single perithecium and incubated for 13 days at 26°C.

GRAPH I-THE EFFECT OF VARYING QUANTITIES OF SUGARS UPON THE GROWTH OF LILLIPUTIA MARGARITACEA USING POTASSIUM NITRATE AS THE NITROGEN SOURCE. MEAN DIAMETER OF COLONIES AFTER EIGHT DAYS AT 26° C.



GRAPH 2- THE EFFECT OF VARYING QUANTITIES OF SUGARS UPON THE GROWTH OF LILLIPUTIA MARGARITACEA USING α-ASPARAGINE AS THE NITROGEN SOURCE. MEAN DIAMETER OF COLONIES AFTER EIGHT DAYS AT 26° C.

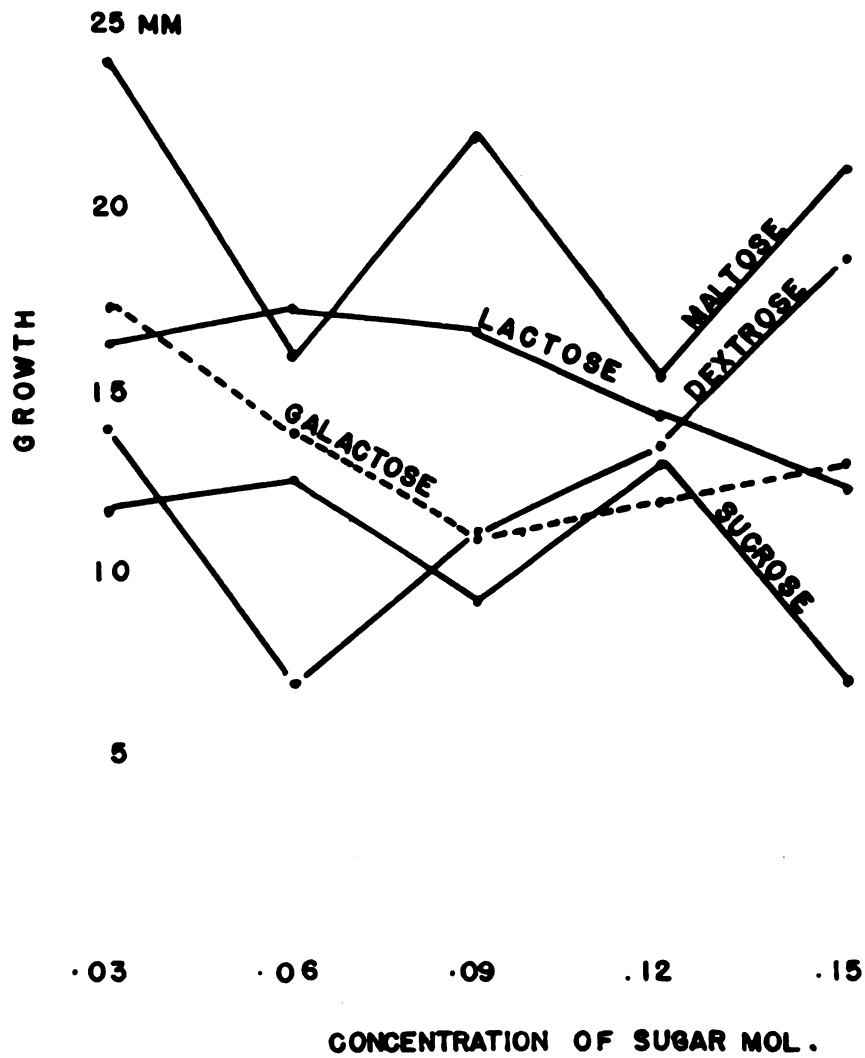


TABLE VI. Eight days' growth of *Lilliputia margaritacea* at 26°C on Lactose Medium with KNO_3 or a-asparagine, in mm. Expressed as an Average Diameter of Three Plates.

Gr. of lactose per liter of medium	Molar concentration of lactose	Gr. of carbon supplied per liter of medium	Growth in mm. KNO_3=N Source	Fruiting	Growth in mm. a-asparagine=N source	Fruiting
10.27	.03	4.32	16.3		16.3	
20.53	.06	8.64	15.6		17.3	
30.80	.09	12.96	14.6	None	16.6	None
41.06	.12	17.27	8.0		14.3	
51.32	.15	21.59	6.6		12.3	

TABLE VII. Eight days' growth of *Lilliputia margaritacea* at 26°C on Galactose Medium with KNO_3 or a-asparagine, in mm. Expressed as an Average Diameter of Three Plates.

Gr. of galactose per liter of medium	Molar concentration of Galactose	Gr. of carbon supplied per liter of medium	Growth in mm. KNO_3=N source	Fruiting	Growth in mm. a-asparagine=N source	Fruiting
5.40	.03	2.15	12.6		17.3	
10.80	.06	4.33	14.6		13.8	
16.21	.09	6.48	16.3	None	11.0	None
21.61	.12	8.63	12.5		12.0	
27.01	.15	10.79	11.6		15.0	

TABLE VIII. Eight days' growth of Lilliputia margaritacea at 26°C on Glucose Medium with KNO_3 or a-asparagine, in mm. Expressed as an Average Diameter of Three Plates.

Gr. of Glucose per liter of medium	Molar concentration of Glucose	Gr. of carbon supplied per liter of medium	Growth in mm. KNO_3=N source	Fruiting	Growth in mm. a-asparagine=N source	Fruiting
5.95	.03	2.15	4.1		14	
11.89	.06	4.33	7.6		7	
17.84	.09	6.48	7.0	None	11.3	None
23.79	.12	8.63	5.0		13.6	
29.74	.15	10.79	8.0		13.6	

TABLE IX. Eight days' growth of Lilliputia margaritacea at 26°C on Sucrose Medium with KNO_3 or a-asparagine, in mm. Expressed as an Average Diameter of Three Plates.

Gr. of Sucrose per liter of medium	Molar concentration of Sucrose	Gr. of carbon supplied per liter of medium	Growth in mm. KNO_3=N source	Fruiting	Growth in mm. a-asparagine=N source	Fruiting
10.27	.03	4.32	8.3		11.6	
20.53	.06	8.64	6.6		12.6	
30.80	.09	12.96	3.6	None	9.3	None
41.06	.12	17.27	8.6		13.0	
51.32	.15	21.59	6.0		7.0	

TABLE X. Eight days' growth of Lilliputia margaritacea at 26°C on Maltose Medium with KNO_3 or a-asparagine, in mm. Expressed as an Average Diameter of Three Plates.

Gr. of Maltose per liter of medium	Molar Concentration of maltose	Gr. of carbon supplied per liter of medium	Growth in mm. KNO_3-N source	Fruiting	Growth in mm. a-asparagine-N source	Fruiting
10.27	.03	4.32	19.6		24	conidia
20.53	.06	8.64	21.6		16	
30.80	.09	12.96	22.0	None	22	found in
41.06	.12	17.27	20.6		15.3	
51.32	.15	21.59	15.6		21	all plates.

Maltose	3.08 gms per 100cc.	Results:
$MgSO_4$	.123 " " "	.122gms. of $(NH_4)_2HPO_4$
$(NH_4)_2HPO_4$	.122 " " "	produced 16mm. of
KH_2PO_4	.272 " " "	growth; mycelium
Agar	1.5%	sparse, conidia and
Distilled water	100cc.	perithecia absent.
Trace Fe		

Ammonium nitrate

The following medium was prepared in triplicate, inoculated with a perithecium and incubated at 26°C for 13 days.

Maltose	3.08 gms. per 100cc.	
$MgSO_4$	.123 " " "	
NH_4NO_3	.03 " " "	=.230 gms. N per liter
KH_2PO_4	.272 " " "	
Agar	1.5%	
Water	100cc.	Results:
Trace Fe		No growth.

IV, B. Light

A plate of oat agar containing the fungus which had grown the diameter of the plate, was left in a dark cupboard for a few days. When removed, perithecia were found in great abundance. This was the first indication that light was not necessary for fruiting.

In order to keep all plates at approximately the same temperature, a large petri dish container was packed with cotton leaving enough room up next to the container cover for 6 Petri plates. Those plates exposed to light were placed on top of the Petri dish container 2 feet under a 60 watt lamp for 10 days. Those exposed to darkness were left inside the Petri dish container and those exposed to intermittent light were placed on top of the container cover for 12 hours and inside the container for 12 hours alternately. The container was opened only when placing the intermittent light plates from light in darkness and in light again. This was done in a darkroom.

The agar used, together with growth, reproduction results, and

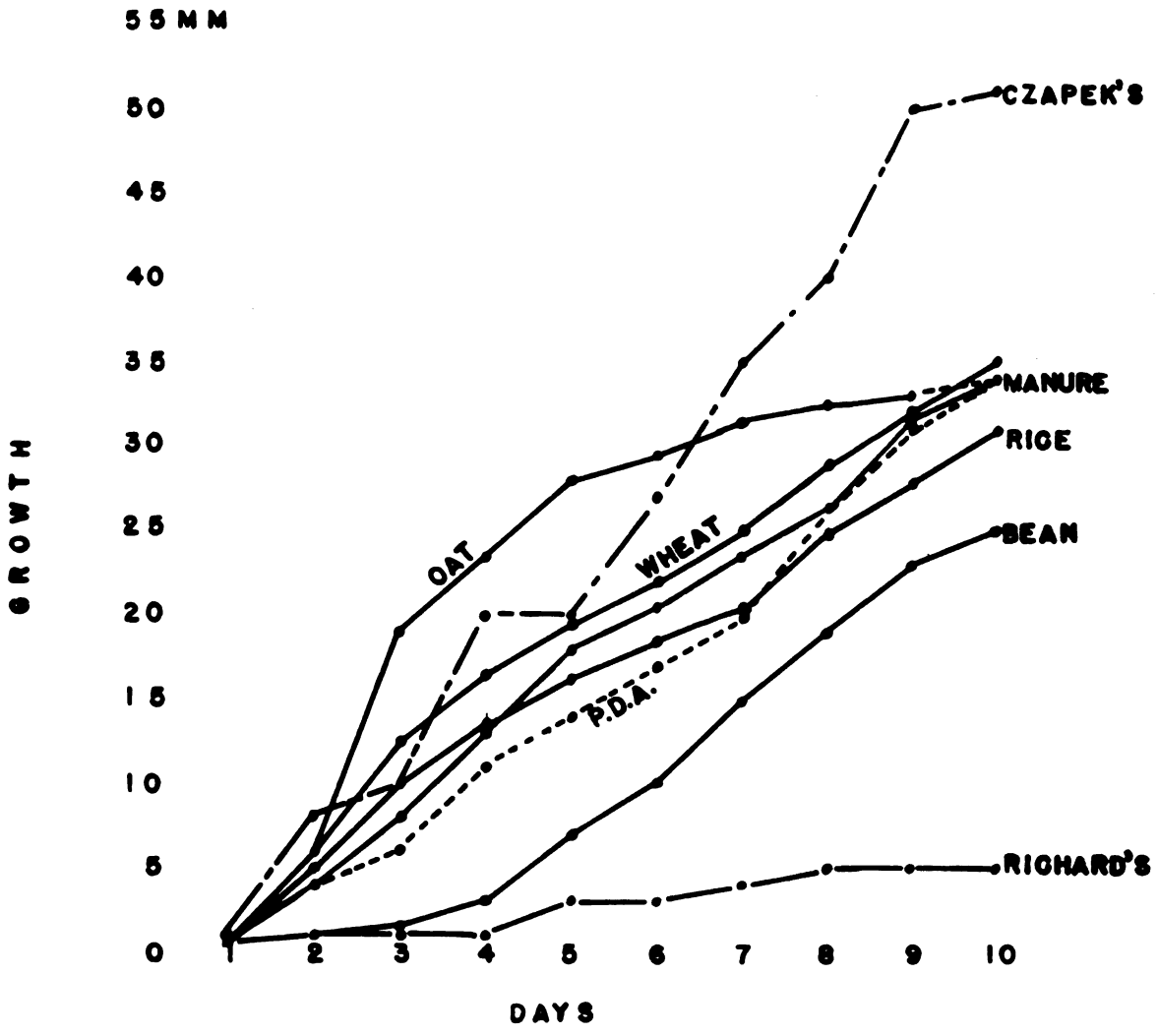
TABLE XI. Ten Days' Growth of Lilliputia margaritacea at 26°C at Various Light Intensities, on Different Media, as Represented by Average Diameters of 3 Plates in mm.

Media	Light	Intermittent Light	Darkness
Wheat agar pH 7.2	35	34	36
Rice agar pH 6.2	31	32	31
Manure (3%) agar pH 6.9	34	32	30
Oat agar pH 7.0	34	66	90
Potato dextrose agar pH 5.7	34	32	35
Sterile whole manure	Measurements were not made from test tubes.		
Carrot plugs	10	10	10
Bean Pod agar pH 4.9	25	23	30
Red Clover stems	0	0	0
Czapek's synthetic medium pH 6.8	51	51	57

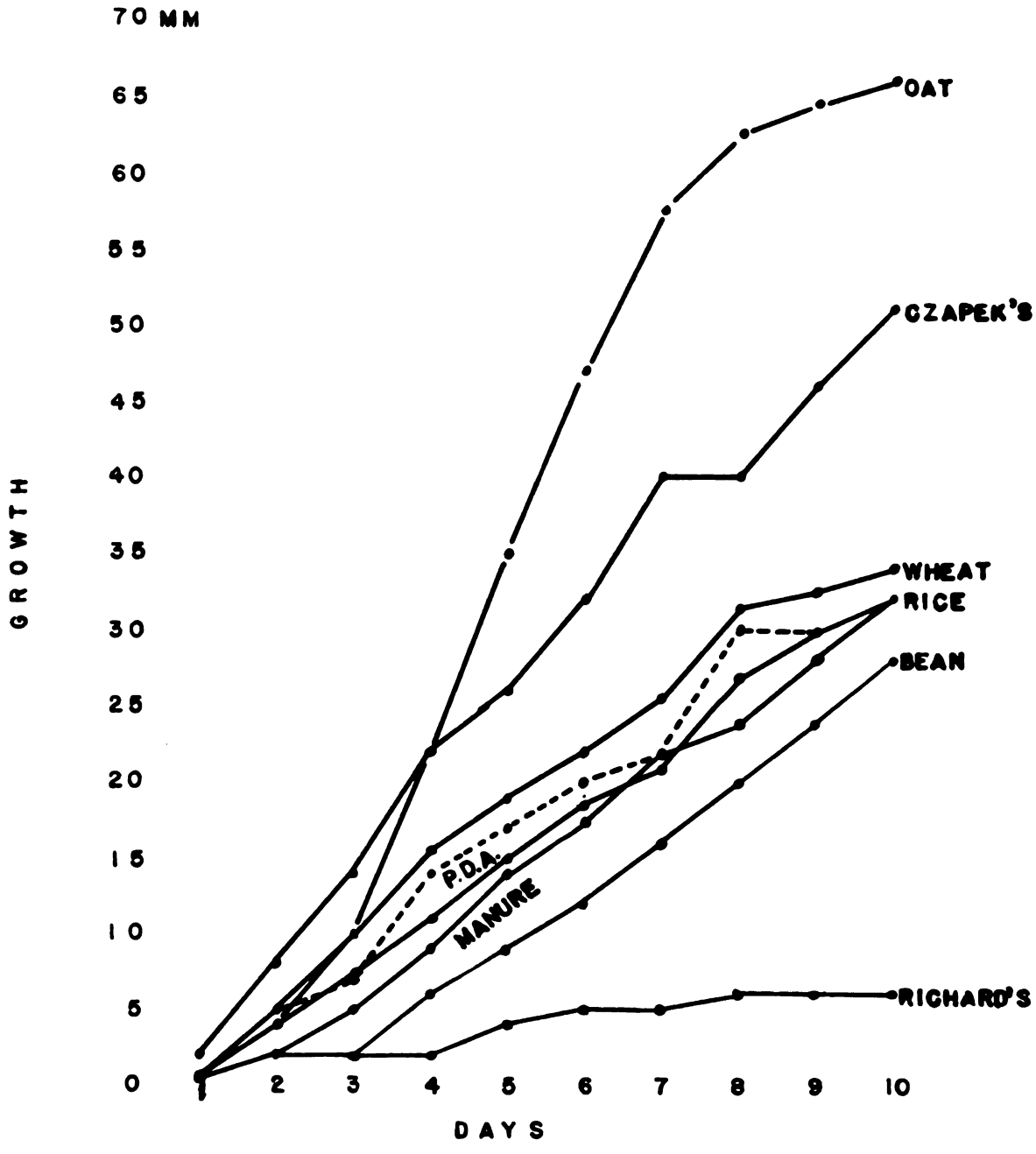
TABLE XII. The Effect of Various Light Intensities on the Fruiting of Lilliputia margaritacea in Various Media as Represented by Macroscopic Observations (10 days' growth at 25°C)

Media	Light	Intermittent Light	Darkness
Wheat agar	Perithecia & conidia formed 5 days after inoculation	Perithecia; 3 days after inoc. Conidia; 3 days after inoc.	Perithecia & Conidia; 5 days after inoc.
Rice Agar	Perithecia; 9 days after inoc. Conidia; 5 days after inoc.	Perithecia; 3 days after inoc. Conidia; 3 days after inoc.	Perithecia; 5 days after inoc. Conidia; absent
3% Manure Agar	Perithecia & conidia absent	Perithecia & conidia sparse	Perithecia & conidia sparse.
Oat Agar	Perithecia; 10 days after inoc.	Perithecia; 3 days after inoc.	Perithecia and conidia; 5 days after inoc.
Potato dextrose agar	Perithecia & conidia absent	Conidia; 5 days after inoc.	Conidia; 5 days after inoc.
Sterile whole manure	Perithecia; 10 days after inoc. Conidia; 5 days after inoc.	Perithecia; 7 days after inoc. Conidia; 5 days after inoc.	Perithecia; 7 days after inoc. Conidia; 5 days after inoc.
Carrot plugs	Perithecia & conidia absent	Perithecia & conidia absent	Perithecia & conidia absent
Bean pod agar	Perithecia & conidia absent	Conidia; 10 days after inoc.	Conidia; 9 days after inoc.
Red clover stems	----------no growth----------		
Czapek's synthetic medium	Conidia; 7 days after inoc.	Conidia; 5 days after inoc.	Perithecia & conidia; absent*

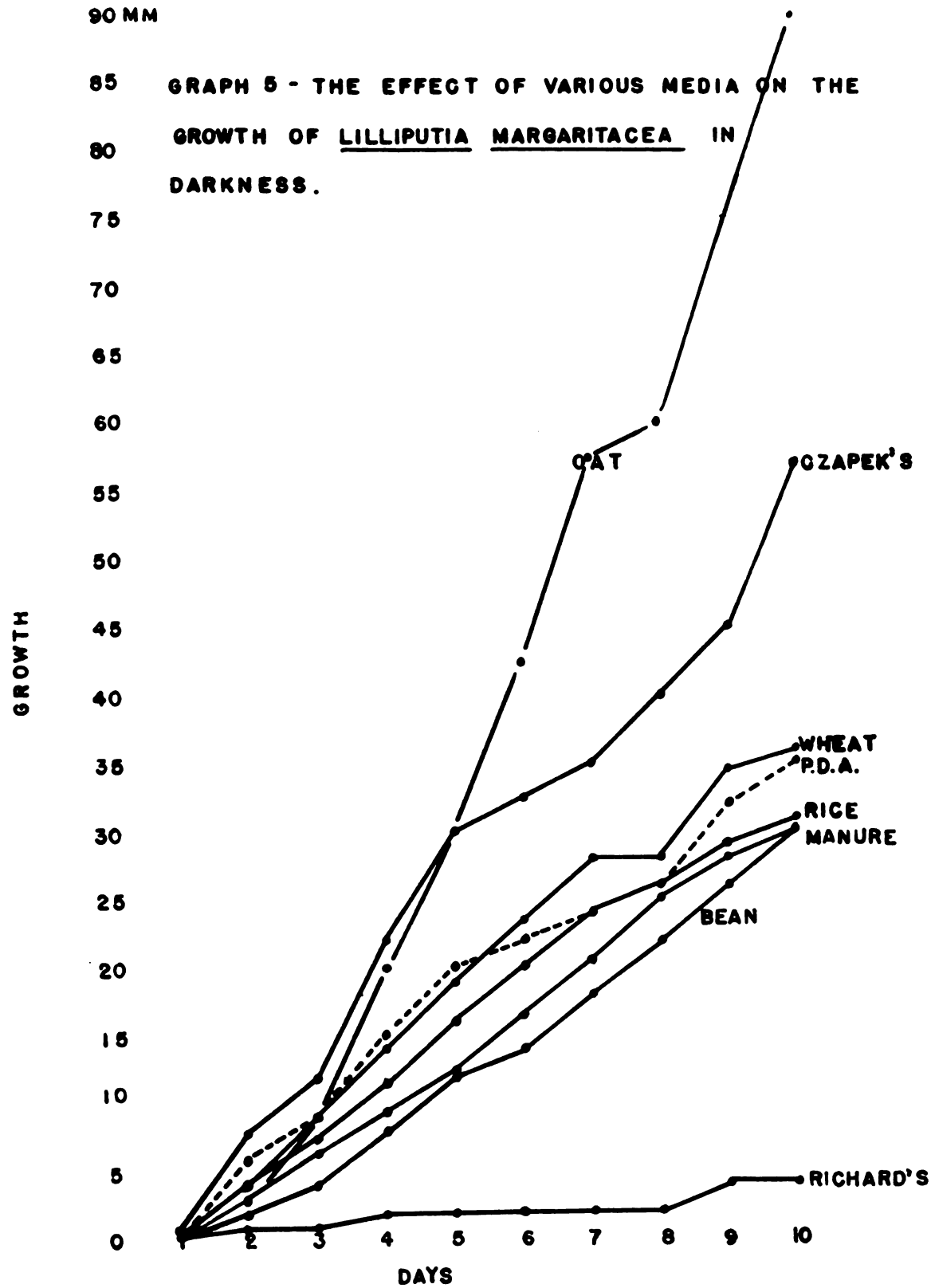
* If perithecia and/or conidia were not found within a ten day period, the author does not mean to state that they would not have been formed at all. On the contrary, in many cases both stages of the fungus did show up in 15-20 days, but the purpose of this section was to find a medium which would produce both stages as early as possible.



GRAPH 3 - THE EFFECT OF VARIOUS MEDIA ON THE GROWTH OF LILLIPUTIA MARGARITACEA IN LIGHT.



GRAPH 4 - THE EFFECT OF VARIOUS MEDIA ON THE GROWTH OF LILLIPUTIA MARGARITACEA IN INTERMITTENT LIGHT.



GRAPH 5 - THE EFFECT OF VARIOUS MEDIA ON THE GROWTH OF LILLIPUTIA MARGARITACEA IN DARKNESS.

microscopic measurements of spores are given in TABLES XI to XIII and GRAPHS 3-5.

The size of conidia was found to vary under different light intensities and on different media. This is shown on TABLE XIII.

Lilliputia margaritacea produces three types of conidia; elliptical, ovate, and spherical. Average measurements are as follows:

elliptical 2.4-2.8 x 7.0-7.7 microns

spherical 4.3-12.7μ

ovate 3.2-5.8 x 5.7-7.3μ

These measurements represent the extreme in length and width of each type of conidia resulting from growth in the media listed in TABLE XII.

Elliptical conidia were most prevalent.

IV, B, 3. Effect of Temperature and pH on Growth and Reproduction

To find the combined effects of varied hydrogen ion concentrations and temperatures on growth and reproduction of Lilliputia margaritacea, wheat and rice agars were prepared, autoclaved, and adjusted aseptically (15) with a Beckman pH meter to pH's 5.6, 6.0, 7.1, 7.4, and 8.0. Three Petri plates of each medium, for each pH were inoculated- each with a perithecium, and placed into incubators previously adjusted to 14, 18, 24, 26, 28, 30, 32, and 34°C. Growth measurements were made everyday for 10 days and fruitification results were recorded (TABLE XIV).

Data in TABLE XIV ~~was~~ were compiled in the summer of 1948. This experiment was duplicated in the fall of 1949, using Fries modified synthetic agar plus .5% yeast extract in place of rice agar. As shown in TABLES XIV and XV the results obtained on wheat agar in 1949 varied greatly from those obtained on the same agar in 1948. This variation may have been due to a difference in the variety of wheat used in the two experiments.

The optimum temperatures and pH's (TABLE XVI) are based on the following conditions which were considered and listed in order of their

TABLE XIII. Effect of Light, Intermittent Light, and Darkness on Conidial Size. Measurements in μ.

Types of Spores	P. D. A.			Wheat Agar		
	Light	Int. Light	Darkness	Light	Int. Light	Darkness
1. Elliptical			2.4 X 7.3			
2.						
3. Ovoid		3.7 X 6.6	4.3 X 5.7	4 X 6.5	3.4 X 6.5	3.9 X 6.6
4.					6.7 X 6.7	7.3 X 7.3
5.				9.1 X 9.1		9.0 X 9.0
6. Spherical					11.6 X 11.6	
7.				13.7 X 13.7		
	Oat			Rice		
1. Elliptical						
2.	4.3 X 7.2	4.5 X 7.2	4.3 X 7.2		4.4 X 7.2	
3. Ovoid				3.2 X 6.1	3.8 X 6.2	
4.	7.2 X 7.2		7.2 X 7.2		8.6 X 8.6	
5.	9.2 X 9.2		9.2 X 9.2			
6. Spherical						
7.						
	Manure Ag			Czapek's		
1. Elliptical				2.5 X 7.7	2.6 X 7.2	
2.		4.3 X 7.3	5.3 X 6.7			
3. Ovoid						
4.						
5.						
6. Spherical						
7.						
	Sterilized whole manure					
1. Elliptical	2.3 X 7.3	2.7 X 7.0	2.3 X 7.0			
2.			3.6 X 7.2			
3. Ovoid						
4.			4.3 X 7.0			
5.						
6. Spherical						
7.						

TABLE XIV. Effects of Hydrogen Ion Concentration, Temperature, and Media on the Growth of Lilliputia paritricea. (Measurements made after 10 days' growth.)

Wheat agar

Temperature	pH					
	5.7	6.2	6.5	7.0	7.4	8.0
14°C	3 mm*	17	13	15	21P	21(P)
18	27 (G)	35 (G)	40 (G)	36 (G)	36	39 (P)
24	35 (G)	45 (G)	45 (GP)	51	47 (GP)	46 (GP)
26	40 (G)	37 (GP)	55 (GP)	37 (G)	63 (GP)	57 (GP)
28	43 (G)	44 (G)	56 (GP)	62 (G)	62 (G)	55 (G)
30	28	20	35	34	34	33
32	0	0	sl.t.	sl.t.	0	0
34	0	0	0	0	0	0

Rice agar

Temperature	pH				
	5.7	6.2	7.0	7.4	8.0
14°C	14	15	15	15	19
18	21 (G)	32 (G)	39 (G)	35 (G)	33 (G)
24	34 (G)	36 (G)	40 (G)	43 (G)	50 (G)
26	42	47 (GP)	46 (GP)	55 (G)	56 (G)
28	37 (G)	40 (G)	40 (G)	51 (G)	30 (G)
30	27	31 (G)	33	33	32 (G)
32	13	5	5	3	4
34	tr.	tr.	tr.	0	0

* Growth in mm. Mean diameter of three plates in each case.
P- Perithecia produced
G- Conidia produced
sl.t.- Slight trace
tr.- Trace

TABLE IV. Effects of Hydrogen Ion Concentration, Temperature, and Medium on the Growth and Fruiting of Lilliputia terricolae. (Measurements made after 10 days' growth.)

Temperature	Wheat agar pH				
	6.0	6.5	7.0	7.4	8.0
22°C	45 (G)*	51 (GF)	55 (GF)	64 (GF)	60 (GF)
24	48 (GF)	52 (GF)	48 (GF)	56 (GF)	68 (GF)
26	54 (F)	65 (F)	60 (F)	71 (F)	74 (F)
28	53 (GF)	66 (F)	70 (F)	64 (GF)	78 (GF)
30	60	70	83	72	80

Temperature	Fries modified synthetic plus .5% yeast extract pH				
	6.0	6.5	7.0	7.4	8.0
22°C	57 (F)	58 (F)	68 (F)	69 (F)	59 (F)
24	70 (F)	73 (GF)	75 (F)	75 (GF)	63 (GF)
26	55 (F)	55 (GF)	75 (G)	85 (G)	82 (GF)
28	67 (GF)	68 (GF)	76 (G)	80 (G)	72 (G)
30	49 (GF)	61 (G)	72 (G)	90 (G)	82 (G)

* Growth in mm. Mean diameter of three plates in each case.
F- Perithecia produced.
G- Conidial produced.

importance when determining these optima.

1) The abundance of perithecia and conidia produced.

2) The size and thickness of the colony after growth period.

3) The speed with which perithecia and conidia were produced.

4) The speed of maturation of the asci.

IV, C. Effect of Various Factors on the Taxonomic Characters of the Fungus

1. Nutrition, Temperature, and Hydrogen Ion Concentration

Synthetic and organic media

Measurements of various fungus structures were made from cultures grown on wheat agar and modified Fries synthetic agar plus .5% yeast extract. Each medium was divided into five, 500cc. aliquots and one aliquot adjusted to each of the following pH values: 6.0, 6.5, 7.0, 7.4, and 8.0. Approximately 15cc. of agar were poured into each of 15 Petri dishes which were subsequently inoculated. Three dishes were incubated at each of the following temperatures- 22, 24, 26, 28, and 30°C.

The measurements obtained are summarized in TABLES XVI to XLV.

TABLE XVI. Optimum Temperatures for Growth of Lilliputia margaritacea on Various Agars at Varied Hydrogen Ion Concentrations.

Hydrogen-Ion Concentrations	Optimum temperatures: Rice agar	Wheat agar	
5.7	23°C	23°C	The optimum temperature, for growth and reproduction,
6.2	26	26	and pH on Rice agar is 26°C at pH 6.2.
6.5	--	26-23	
7.0	26	23	The optimum temperature, for growth and reproduction,
7.4	26	26	and pH in Wheat agar is 26°C at pH 7.4.
8.0	26	26	
	Wheat agar	Fries modified synthetic	
6.0	24°C	23°C	The optimum temperature, for growth and reproduction,
6.5	24	24	and pH in Wheat agar is 24°C at pH 7.4.
7.0	24	23	
7.4	24	24	The optimum temperature, for growth and reproduction,
8.0	26	26	and pH in Fries modified synthetic plus .5% yeast extract is 24°C at pH 6.5.

TABLE XVII. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements in μ Made After 11 days Growth.

Structures measured	Malt agar pH 6.0 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical	2.7 X 7.0	2.7 X 7.0		2.7-3.6 X 7.3-8.6	
oval	3.4 X 6.8	3.4 X 6.8		3.6 X 6.8	
spherical	4.3-7.7	4.7	No	4.3	
Conidiophores	129-253	253	Conidia	200-250	
top	3.6-12.9	3.6	produced	3.6	
bottom	9.5-12.9	12.9		10.7-12.9	No
Branches	4.3 X 21-25	3.2 X 17.2-30		4.3-5.5 X 17.2-25.8	Conidia
Metulae	3.4 X 15.6	2.5-4.5 X 8.6-17.2		3.6-4.3 X 17.2	or
Phialides	2.7 X 13.0	2.1 X 25.3		2.5-3.2 X 17.2-20.5	Perithecia
Perithecia					produced
hairs	Not	Not	Asci not	Asci not	
wall	mature	mature	differentiated	differentiated	
diam. of gleba					
Asci	30-34.4 X 30-47.3	38-43 X 43-55.9			
Ascospores					
Spines					
Perithecial production	6 days after inoculation	5 days after inoculation	5 days after inoculation	4 days after inoculation	
Conidial production	5 days after inoculation	5 days after inoculation		4 days after inoculation	

TABLE XVIII. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements in μ Made after 11 days Growth.

Structures measured	Wheat agar pH 6.5 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical	2.7 X 7.0	none			
oval	3.4 X 5.8	3.2-34 X 6.8-8.0			
spherical	4.3	4.3			
Conidiophores	239-383	274-392	No	No	Conidia
top	8.1-10.7	7.8-8.6	Conidia	Conidia	and
bottom	11.7-16.6	9-10.0	produced	produced	Perithecia
Branches	4.3 X 23.6-30	4.3 X 20-30			not
Metulae	3.2-3.4 X 8.6-21.5	3.6 X 17.2			produced
Phialides	1.5-2.3 X 17.2-23.6	2.3 X 21.5			
Perithecia	450-700	400-700			
hairs	yes	yes			
wall	73.1-88	77.4-64.5	Asci	Asci	
diam. of gleba	352-627	322-622	not differentiated	not differentiated	
Asci	40.3 X 47.3	43-47.3 X 39-64.5			
Ascospores	19.3-21.5	21.5-30.1			
Spines	1.7-3.4	1.7-1.9			
Perithecial production	6 days after inoculation	5 days after inoculation	6 days after inoculation	4 days after inoculation	
Conidial production	5 days after inoculation	5 days after inoculation			

TABLE XIX. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements in μ Made after 11 days Growth.

Structures measured	Meat agar pH 7.0 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical	2.3 X 3.3	2.3 X 7.0			
oval	2.3 X 3.3	3.6 X 5.1	No	No	No
spherical	4.3	4.3	Conidia	Conidia	Conidia
Conidiophores	301-470	210-330	produced	produced	or
top	7.9	7.3			Perithecia
bottom	10.7	10.7-13.9			formed
Branches	4.3 X 23.5	3.4 X 21.5-30			
Vetulae	3.4 X 17.2	3.4 X 17.2			
Phialides	3-3.4 X 21.5	2.7-3.2 X 21.5			
Perithecia		600			
hairs	Not Mature	yes	Asci	Perithecia	
wall		70-80	not mature	immature and	
diam. of gleba		470-630		only 2 in number	
Asci	30.1-33.7 X 30-51.6	30.1-33.7 X 51.6-60.2			
Ascospores		21.5			
Spines		1.5-1.7			
Perithecial production	5 days after inoculation	5 days after inoculation	6 days after inoculation	6 days after inoculation	
Conidial production	5 days after inoculation	4 days after inoculation			

TABLE XX. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made in μ after 11 days Growth.

Structures measured	Wheat agar pH 7.4 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical	3 X 3.6	2.3 X 7.0		3.2 X 8.6	
ovoid	3.4 X 5.5	3.4 X 6.8		2.5 X 6.2	
spherical	4.3	4.3	Conidia	none	No
Conidiophores	220-340	240-360	not	130-260	Perithecia
top	5.6	6.4-9.6	produced	10.7	or
bottom	10.7	3.6-12.9		10.7	Conidia
Branches	4.3 X 33.7	4.3 X 21.5-25.8		4.3 X 17.4-21.5	found
Metulae	3.2 X 12.9	3.2 X 3.6-12.9		3.2 X 12.9	
Phialides	2.5 X 12.9	3.2 X 21.5		3.2 X 12.9	
Perithecia		450-700		2.1-3.2 X 12.9	
hairs	Not	yes	Perithecia		
wall	mature	77.4-86.0	immature	Asci not	
diam. of gleba		372-622	and only 2 in number	differentiated	
Asci	25.3-34.4X34.4-33.7	43.0-60.2X47.3-77.4			
Ascospores		21.5-25.8			
Spines		1.2-3.1			
Perithecial production	5 days after inoculation	5 days after inoculation	7 days after inoculation	6 days after inoculation	
Conidial production	5 days after inoculation	4 days after inoculation		4 days after inoculation	

TABLE XXI. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements in μ Made after 11 days Growth.

Structures measured	Wheat agar pH 5.0 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical	3.4 X 9.0	3.0 X 7.7		3.2 X 8.6	
oval	3.4 X 6.2	4.3 X 6.2	Conidia	63.6-4.3X5.3-6.3	
spherical	4.3	none	not		No
Conidiophores	100-340	220-360	formed	200-400	Conidia
top	3.6	4.3-3.6		6.4-9.6	or
bottom	10.7-12.9	11.9-17.9		8.6-10.7	Perithecia
Branches	2.7-4.3X52.2	4.3X30.1		4.3X12.5	produced
Metulae	2.3-3.2X3.6-12.9	2.3X10.7		2.3X14.9	
Phialides	2.3X21.5	2.1X21.5		2.1X12.9	
Perithecia					
hairs	Perithecia	Perithecia	Perithecia	Perithecia	
wall	not	not	very	not	
diam. of gleba	mature	mature	sparse	mature	
Asci					
Ascospores					
Spines					
Perithecial production	6 days after inoculation	5 days after inoculation		4 days after inoculation	
Conidial production	5 days after inoculation	5 days after inoculation		4 days after inoculation	

TABLE XXII. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made in μ after 11 Days Growth.

Structures measured	Fries Modified Synthetic pH 6.0 (4.5% yeast extract) Temperature 22°C	24°C	26°C	28°C	30°C
Spores					
elliptical				3.8 X 8.6	2.0 X 7.6
ovoid				3.8 X 6.8	3.7 X 6.9
spherical	Conidia	Conidia	Conidia	4.3	3.4-4.3
Conidiophores	absent	absent	absent		
top				150	255
bottom				3.8-17.0	6.8-9.8
base				3.8-15.0	10-14.5
Branches				4.3 X 12.4	3.4 X 12.5
Metulae				4.3 Y 8.6	2.8-3.4 X 15.0
Phialides				1.7-2.1 X 15	2.1 X 12.9
Perithecia	400-300	400-300	401-600	400-450-600	
hairs	yes	yes	yes	yes	
wall	77.4	87.5-94.6	78.1	77.4	Perithecia 2 in number
diam. of glebu	570	393-470	353-537	382-533	
Asci	48-51.6X47.8-58.3	60.2 X 77.4	48-47.8X48-51.6	50.1-51.2X50.1-58.0	
Ascospores	21.5-27.9	20.6-25.8	19.3-23.6	17.2-21.5	
Spines	2.1-2.5	1.9-4.9	1.5-2.7	1.3-2.5	
Perithecial production	5 days after inoculation	4 days after inoculation	4 days after inoculation	4 days after inoculation	
Conidial production				6 days after inoculation	6 days after inoculation

TABLE XXIII. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made 11 Days after Growth. Measurements made in μ.

Structures measured	Tries modified synthetic plus .5% yeast extract pH 6.5 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical		2.3 X 3.6	2.3 X 3.6	2.3 X 7.7	2.1 X 7.0
ovoid		3-3.4 X 6.2	3.4 X 6.4	3.2-3.4X6.4	3.4-3.9X7.0
spherical	Conidia	none	4.3	4.3	4.5-4.9
Conidiophores	absent	200	100-200	260	270
top		7.9-3.6	3.6	3.6	7.0-9.3
bottom		3.6	9-3.6	12.9	11-13.7
Branches		3.4 X 21.5	3.4X17.2-21.5	3.4X21.5	4.3 X 23
Retulae		3.2 X 3.6	3.2 X 17.2	3.2 X 3.6	3.3-3.3X21.5
Phialides		2.1 X 15	2.3 X 17.2	2.1 X 12.5	2.1 X 9.6
Perithecia	600-750	500-700	500-700	400-600	
hairs	yes	yes	yes	yes	
wall	77.4	64-73	64-73	63-77.4X322-537	
diam. of gleba	528-672	427-636	427-638		Perithecia
Asci	30-54X40-56	37.9X54	47.3-30.2X60.2-64.5	30-51.6X50-70	absent
Ascospores	21.5-25.8	21.5-25.3	21.5-25.8	21.5	
Spines	1.5-1.9	1.5-2.5	1.5-2.5	1.5	
Perithecial production	6 days after inoculation	4 days after inoculation	4 days after inoculation	4 days after inoculation	
Conidial production		7 days after inoculation	8 days after inoculation	10 days after inoculation	6 days after inoculation

TABLE XXIV. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made 11 days after Growth. Measurements made in μ.

Structures measured	Fries modified synthetic plus .5% yeast extract pH 7.0 Temperature				
	20°C	24°C	26°C	28°C	30°C
Spores					
elliptical			3.2 X 7.9	2.7 X 8.6	2.5 X 8.6
ovoid			2.6 X 7.0	4.3 X 6.4	2.5 X 6.6
spherical	Conidia	Conidia	3.0-5.3	4.3-6.4	4.3
Conidiophores	absent	absent	156-274	200-280	280-300
top			3.6-8.6	8.6	6.6-8.6
bottom			3.6-12.9	10.7	9.6-10.7
Branches			3-4.3X12.9-21.5	4.3 X 21.5	4.3 X 19.3
Metulae			2.7-3.4X10.7-12.9	3.4 X 8.6	3.4 X 8.6
Phialides			2.1 X 12.9	2.3 X 12.9	2.3 X 12.9
Perithecia	400-700	450-750			
hairs	yes	yes			
wall	70-73.4	77.4-31.7	Perithecia immature	Perithecia sparse	Perithecia sparse
diam. of gleba	321-630	363-672			
Asci	34.4-47.7X43-47.3	33.9-47X56-65			
Ascospores	21.5	19.3-25.8			
Spines	1.5-1.7	1.5-1.7			
Perithecial production	7 days after inoculation	6 days after inoculation	6 days after inoculation	6 days after inoculation	6 days after inoculation
Conidial production			7 days after inoculation	6 days after inoculation	6 days after inoculation

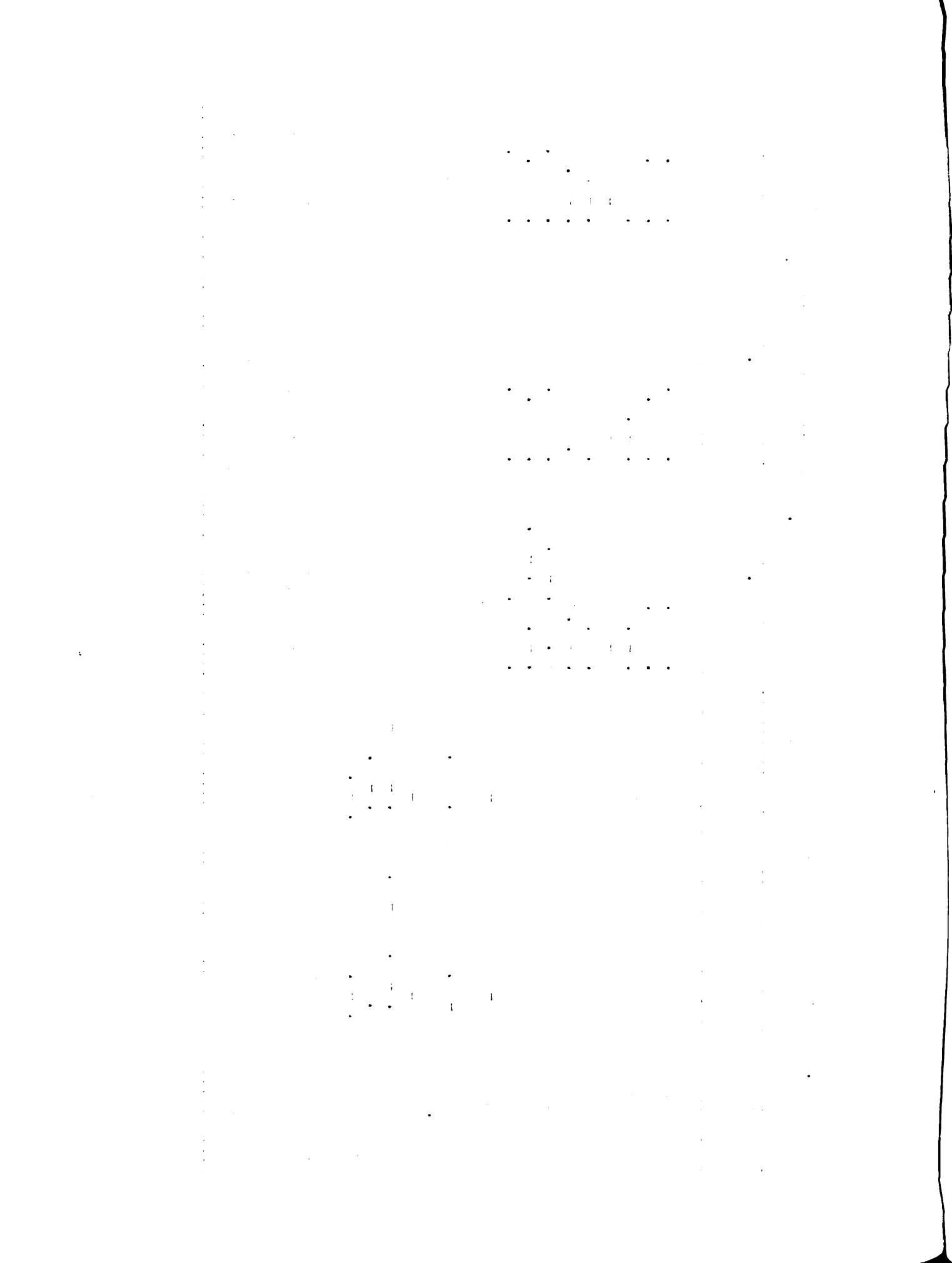


TABLE XXV. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made 11 Days after Growth. Measurements made in μ.

Structures measured	Fries modified synthetic plus .5% yeast extract pH 7.4 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical		3.6-4.3X3.6	2.5-2.7X3.3-3.6	2.5-2.7X3.6	3.6X7.5
ovoid		3.4 X 6.4	2.4-3.6X3.3-3.2	3 X 6.3	3.6X3.3-3.6
spherical	Conidia	4.3-4.9	none	4.3	4.3
Conidiophores	absent	172-274	196-352	250-340	160-340
top		8.6	7.3-11.1	8.6	8.6-12.9
bottom		12.9-17.2	8.6-13.1	10.7	8.6-12.9
Branches		4.3X17.2-21.5	3.6-6.4X12.9-43	3-4.3X8.6	4.3X21.5
Metulae		2.9X17.2	2.3-3.0X14-17.2	3.2X14.9	3.2X15.0
Phialides		2.7X17.2	1.5-2.4X12.9-25.8	2.5X25.8	2.3X21.5
Perithecia					
hairs					
wall					
diam. of gleba	Perithecia immature	Perithecia immature	Perithecia absent	Perithecia absent	Perithecia absent
Asci					
Ascospores					
Spines					
Perithecial production	3 days after inoculation	6 days after inoculation			
Conidial production		7 days after inoculation	6 days after inoculation	6 days after inoculation	6 days after inoculation

TABLE XXVI. Effects of Environmental Conditions on the Growth of Lilliputia margaritacea as Represented by Microscopic Measurements Made 11 Days after Growth. Measurements made in μ.

Structures measured	Fries modified synthetic plus .5% yeast extract pH 6.0 Temperature				
	22°C	24°C	26°C	28°C	30°C
Spores					
elliptical		3.2 X 8.6	3 x 8.6	3.4 X 8.6	3.6 X 6.6
ovoid		3-4.3 X 6.4-7.5	3.3 X 8.6	2.5 X 6.2	3.6 X 8.6
spherical		4.3	4.3	4.3	4.3
Conidiophores	Conidia				
top	absent	94.6-280	280-340	180-300	200-300
bottom		12.9	10.7	8.6-9.6	8.6
base		10.8	17.2	10.7	12.9
Branches		4.3 X 34.4	4.3 X 21.5	4.3 X 21.5-30.1	4.3 X 21.5
Vetulae		3.2 X 12.9-17	3 X 17.2	3.4 X 12-15	3.4 X 8.6
Phialides		2.4 X 13-25.8	5 X 25.3	3 X 17.2	3.4 X 17-21.5
Perithecia					
hairs					
wall					
diam of gleba	Perithecia immature	Perithecia sparse and immature	Perithecia sparse and immature	Perithecia absent	Perithecia absent
Asci					
Ascospores					
Spines					
Perithecial production	6 days after inoculation	10 days after inoculation	6 days after inoculation		
Conidial production		8 days after inoculation	6 days after inoculation	8 days after inoculation	6 days after inoculation

Effect of pH, Temperature and Media on Taxonomic Characters of Lilliputia margaritacea Summarized from Data Presented in TABLES XVII to XXVI.

A. Wheat agar

1. Effect of pH

22°C- All types of conidia become larger with an increase in pH.

- Conidiophores are largest at pH 7.0.

24°C- Metulae are longer at pH6-7.

26°C- Conidia absent.

28°C- Conidiophores are larger at pH 3.0.

30°C- Conidia and perithecia absent.

2. Effect of Temperature

pH6.0- Perithecia not mature at all temperatures.

pH6.5- Asci larger at 24°C[1].

pH7.0- Conidia somewhat larger at 22 and 28°C.

- Conidiophores are somewhat smaller at 28°C.

- Branches somewhat longer at 22°C.

pH8.0- No effect.

B. Modified Fries synthetic plus .5% yeast extract agar

1. Effect of pH

22°C- No effect

24°C- Conidiophores slightly larger at pH 8.0.

- Branches and phialides longer at pH 8.0.

26°C- Conidiophore measurements differ.

28°C- Branches, metulae and phialide measurements differ.

30°C- Conidiophore measurements differ.

---

[1] Asci could not all be measured at the same time after maturity and therefore differences are expected.

2. Effect of temperature

pH 6.0- Conidiphores larger at 30°C.

pH 6.5- Differences in metulae and phialides.

pH 7.0- No effect.

pH 7.4- Spherical type spore not present at 26°C.

pH 8.0- Differences in metulae measurements.

C. Comparison of Taxonomic Characters on Fries and Wheat Medium Under Different Conditions of Temperature and pH.

1. Effect of pH.

22°C- conidia absent in Fries.

24°C- phialides larger in wheat-pH 7.4.

26°C- elliptical spores not found in wheat- pH 6.5.

28°C- conidiophores larger in wheat at pH 6.0.

- conidiophores larger in Fries at pH 7.4.

- phialides longer in Fries at pH 7.4.

30°C- no conidia and perithecia found in wheat.

2. Effect of Temperature

pH 6.0- branches somewhat smaller in Fries.

pH 6.5- conidiophores larger at 24°C in wheat agar.

pH 7.0- conidia absent at 22 and 24°C in Fries and 26, 28 30°C in wheat.

pH 7.4- Metulae longer in Fries.

- branches larger in wheat at 28°C.

- conidiophores larger in wheat at 24°C and 28°C than in Fries at the same temperature.

- no spherical spores found in wheat at 28°C, phialides longer in Fries at 28°C.

- Metulae and phialides longer in wheat at 24°C.

D. Overall Variation of Taxonomic Characters as Determined by the above Experiments

Conidia	
elliptical	2.1-4.3 X 6.6-9.0 μ
ovoid	2.4-4.3 X 5.1-8.6 μ
spherical	3.0-6.4 μ
Conidiophores	94.6-450 μ
Branches	2.7-6.4 X 8.6-45 μ
Metulae	2.3-5.3 X 8.6-21.5 μ
Phialides	1.5-3.4 X 8.6-25.8 μ
Perithecia	400-800 μ
Ascospores	17.2-30.1 μ
Asci	25.8-60.2 X 30-86 μ
Spines	1.2-4.9 μ

V. DISCUSSION

When sterile whole manure is inoculated with Lilliputia margaritacea, glistening, hyaline mycelium can be seen after three days growth. The conidial stage is visible on the 5th day when the mycelium is abundant but the individual strands separated. The surface of whole manure is covered with various sizes of numerous, fluid, hyaline droplets just visible to the naked eye. These droplets are found throughout the growing area but are more numerous at the center.

The conidiophores are produced from a much thinner mycelium (PLATE III, Figure 1); they are septate, hyaline, smooth, almost always unbranched and symmetrical. They average 411 by 20 μ in size with a range of 230-431 X 9.4-21μ. The majority of conidiophores are narrower at the upper portion than at the base. They give off two branches (4.3-5.5 X 25.8-34.4μ) which in turn give off 3-4 metulae (3.4-4.3 X 21.5μ) which further branch into 2 phialides (2.2-3.4 X 25.8-43μ) each (PLATE II, Figures 1 & 2). As the culture gets older, asymmetrical branching becomes more pronounced, one to two branches being produced one septum below the first series of branches.

In general, the sterigmata cut off three types of spores; the majority are ellipsoidal (1.6-2.4 X 4.4-10μ). Spherical (6-9μ) and ovoid forms (3.4-3.8 X 6.7-7.8μ) are also produced (PLATE IV, Figure 1). These spores are produced in succession and just before they are cut off, a fluid droplet is formed around the conidial head allowing the older spores to disperse while the younger ones lie side by side on their sides in the droplet. This fluid droplet has the consistency of water until the culture becomes old and/or somewhat dehydrated. When the humidity is high, these droplets are of varied sizes depending on the age of the

conidial apparatus. When the head becomes heavy with spores and fluid, it falls onto the substrate spreading the spores in the immediate area.

In 7-10 days depending on the light intensity, perithecia (400-700μ, mostly 500μwhen mature) are produced--mostly on woody remnants in manure--by the coiling of mycelium which produces a ropy, waxy perithecial wall containing many papillate hairs. The wall is loose and hard at first but becomes more compact and soft at maturity. The wall is 6-9 cells thick (47-73μ), the broadest cells are in the middle layer and the narrowest on either side. When first produced (6th day) the perithecia are light tan and appear pearl-like (PLATE V, Figure 1), but as the ascopores mature within the gleba (324-606μ), their color changes to light brown (at this time the asci are fully mature and the ascospores are hyaline and immature). The mature perithecia finally appear rust colored due to the mature rust colored ascospores (19.3-21.5μ) (PLATE V, Figure 2) showing through the still light brown perithecial wall. In about 15 days the asci appear. They are pyriform and loosely packed, but just before disintegration (17-19th day) of the ascal wall the aculeate, hyaline ascospores are tightly packed (PLATE V, Figure 3) within the ascus. The mature asci are difficult to see because of the thinnes of their walls. They are bulging (24.4-30.1 by 36.5-55.9μ), and have reached their maximum elasticity. Upon disintegration of the asci, the ascospores are still hyaline, and bear aculeate spines about $\frac{1}{2}$ the diameter of the spore in length (PLATE 3, Figure 2). The ascospores at maturity become verrucose to echinulate (1.9-2.3μ) PLATE 5, Figure 2.

Upon comparing the characteristics of this fungus with the descriptions of those listed in the appendix, it was found that our fungus resembles three known species. The similarities and differences between Lilliputia

gaillardii, Gliocladium prolificum, Lilliputia insignis and our fungus are discussed below.

The mature asci of Lilliputia gaillardii are described (5) as oblong. The ascospores are light brown at maturity, but appear white when seen through the perithecium. The mature ascospores of Lilliputia margaritacea are always rust colored at maturity independent of the type of media used for growth. The mature asci are subglobose. The asci of Lilliputia gaillardii as shown by Boudier and Patouillard (5) are loosely packed compared to those of Lilliputia margaritacea (PLATE 5, Figure 3). The asci of Lilliputia gaillardii are small compared to those of our fungus; ascospores of both species have a "warty appearance".

In the description of Gliocladium prolificum, the conidiophores are described as being branched at every septum. This characteristic is not ordinarily exhibited by our fungus. The conidia of Lilliputia margaritacea are mostly elliptical. Those of Gliocladium prolificum are mostly oval. Perithecia associated with G. prolificum were thought to belong to the Perisporiaceae, however from the description of both stages (2) that species appears to be congeneric with L. margaritacea. The color of the polyhedral cells in the perithecial wall, the size and color of the ascospores and the size of the echinulations are like those characteristics of our fungus.

Dennis and Wakefield (9) believe that L. insignis, E. insigne, L. gaillardii, and G. penicillioides are probably the same species. The fungus L. insignis as found in March 1938 at Uxbridge, Middlesex, is described as follows; fruitification glabrous, spherical, white to cream colored, approximately 500μ in diameter, and having a stout wall. These characteristics coincide with those of L. margaritacea, but the

ascospores are small (11-13μ) compared to those of our fungus (12.5-14.5μ). The measurements of L. insignis were made of yellow ascospores within asci and these spores are studded with short blunt warts. The ascospores of L. margaritacea within asci are colorless and have spines ½ the diameter of the spore in length. The spores are dark brown and have short blunt warts only when mature and the asci disintegrated.

A culture of the type species Gliocladium penicillioides was obtained from the "Centraal bureau voor Schimmelcultures" on cherry agar for comparison with L. margaritacea and no fruiting was found. Various media were prepared and inoculated but nothing more than sterile mycelium had been formed.

A variety of synthetic and organic media were prepared and inoculated with L. margaritacea in an attempt to produce both stages of the fungus. Of the synthetic media, Fries modified synthetic (plus 5% yeast extract) produced both stages of the fungus, and perithecia matured within 11 days. Further experimentation is necessary here to produce a true synthetic agar. An approximate analysis of Bacto Yeast Extract was obtained and showed 29 known compounds contained in yeast extract which included vitamins, amino acids and inorganic nitrogenous compounds. These compounds should be broken down into smaller groups and then by a process of elimination, the compound or compounds which add to the growth and reproduction of the fungus, would be known.

Fungi are classified into four groups on the basis of the form of nitrogen which they are able to utilize (39).

1. Utilization of organic nitrogen
2. Utilization of organic and ammonia nitrogen
3. Utilization of organic, ammonia and nitrate nitrogen

4. Capable of fixing elemental nitrogen and utilizing all other forms.

The form of nitrogen necessary for assimilation therefore, depends on the metabolic potentialities of the organism. An attempt to produce a synthetic medium, using Coons agar as a base and substituting varying molarities of sugars in combination with two nitrogen sources (having the same equivalent of nitrogen), failed to produce both stages of the fungus. Conidia were produced only in the maltose-a-asparagine combinations and no perithecia were formed. Had more time been available, here too, nutrients such as vitamins and amino acids could have been added. However, Fries modified synthetic (plus .5% yeast extract) produced both stages of the fungus.

Because Fries modified synthetic (plus .5% yeast extract) produced better growth and reproduction than any other synthetic medium prepared, it was used in a study to show the effects of environmental changes. Wheat was also chosen to represent an organic medium. Fries modified synthetic (plus .5% yeast extract) on the whole produced more fruitification and better growth than did wheat.

Measurements were made of various fungus structures from cultures grown on wheat and Fries modified synthetic (plus .5% yeast extract) which were exposed to various temperatures and pH's. Results showed that of the structures measured, the mature ascospores and spines were the only structures which gave consistent measurements independent of the type of medium used, pH, or temperature. One would deduce then that in this group of fungi (Lilliputia), measurements of ascospores and spines, would be most important in classification and identification.

PLATE I

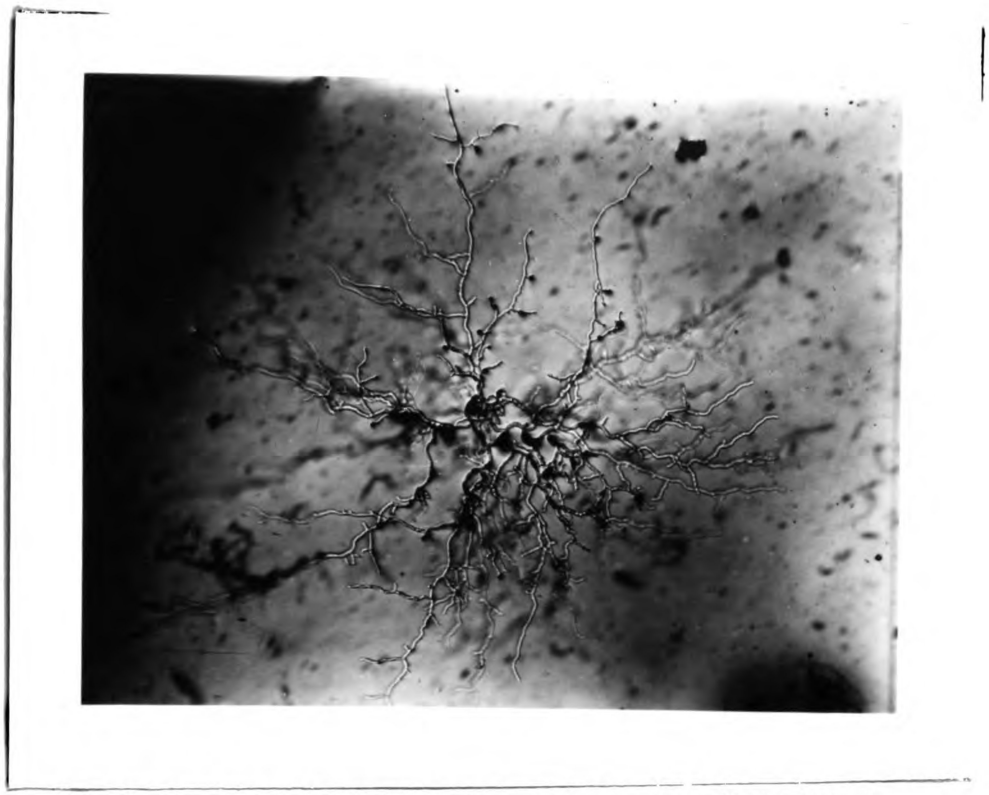


Figure 1. GERMINATION OF A CONIDIUM X100

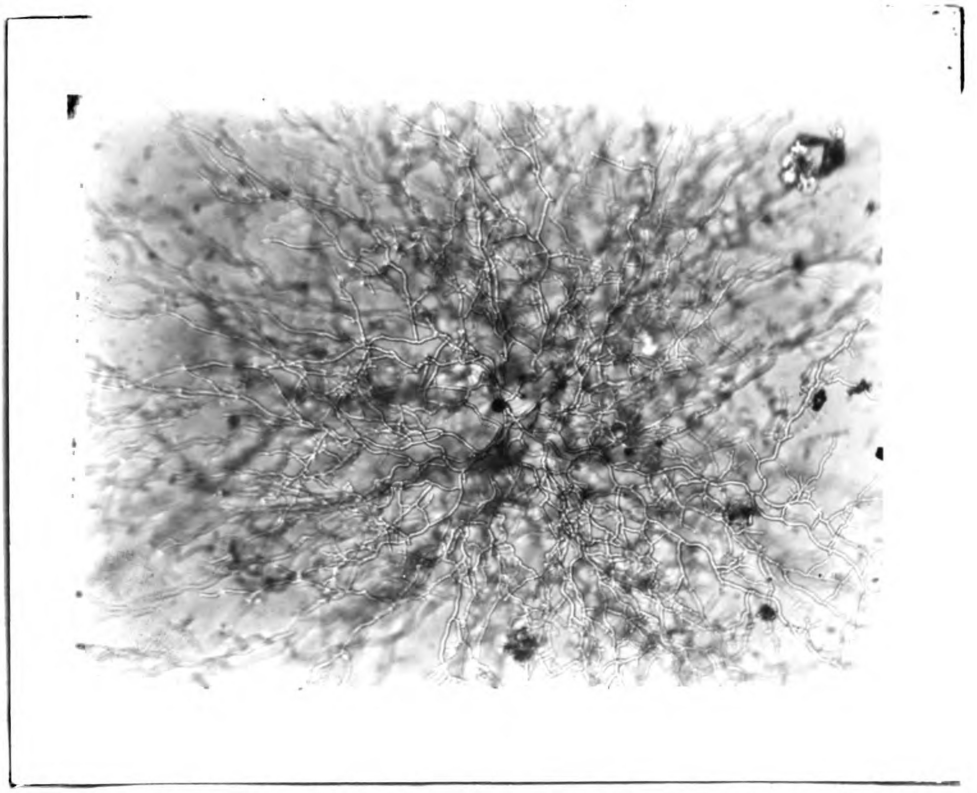


Figure 2. GERMINATION OF AN ASCOSPORE (HCL METHOD) X100

PLATE II.

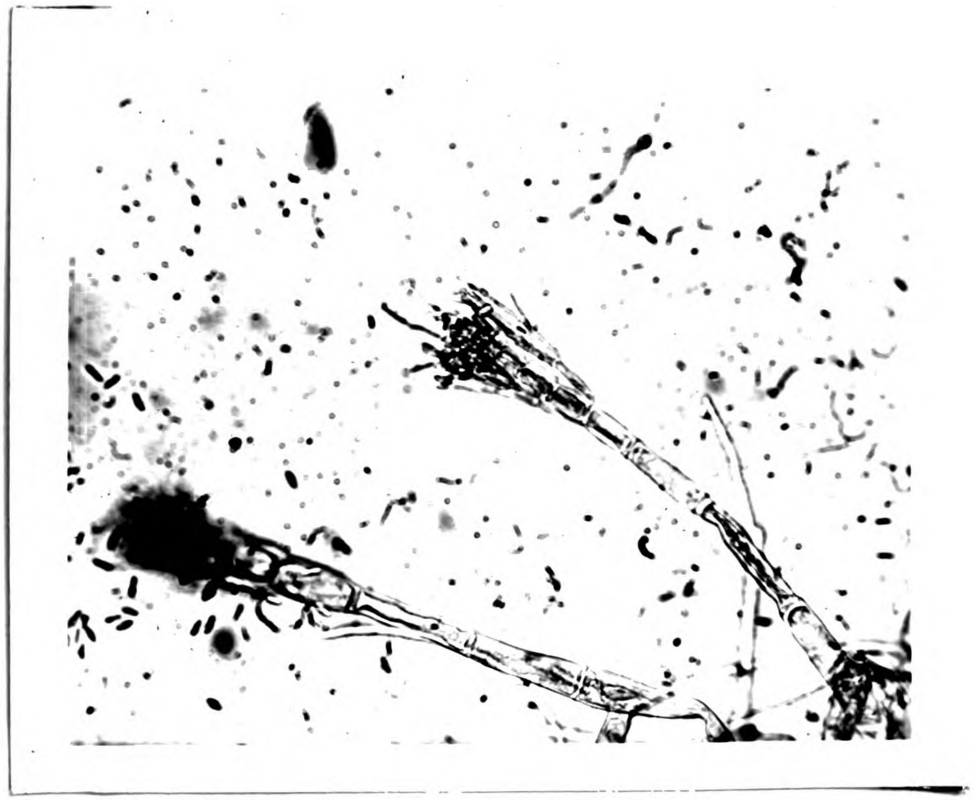


Figure 1. CONIDIAL APPARATUS X150

Figure 2. CONIDIAL APPARATUS X150

PLATE III

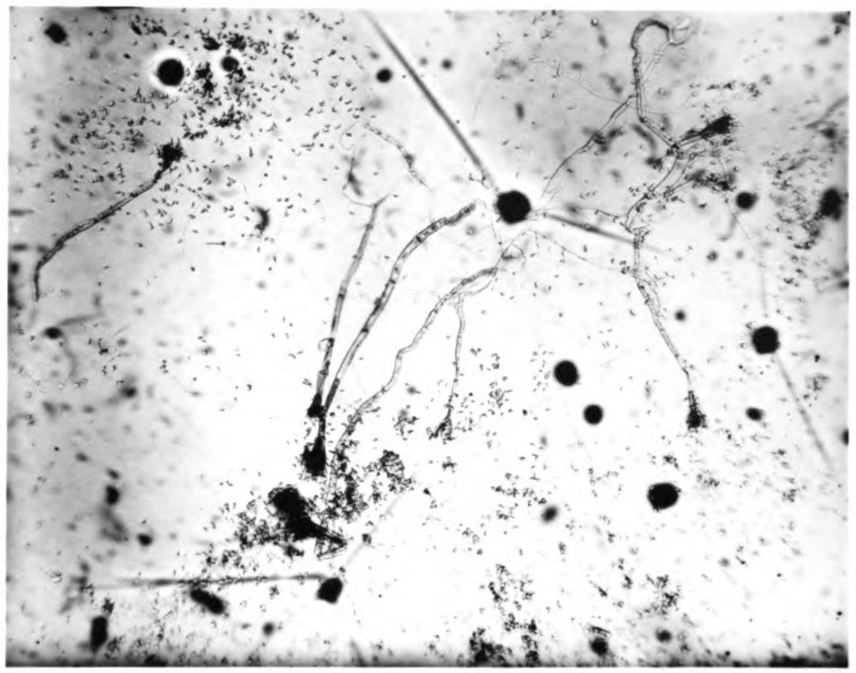


Figure 1. CONIDIOPHORES AND CONIDIAL HEADS. X100

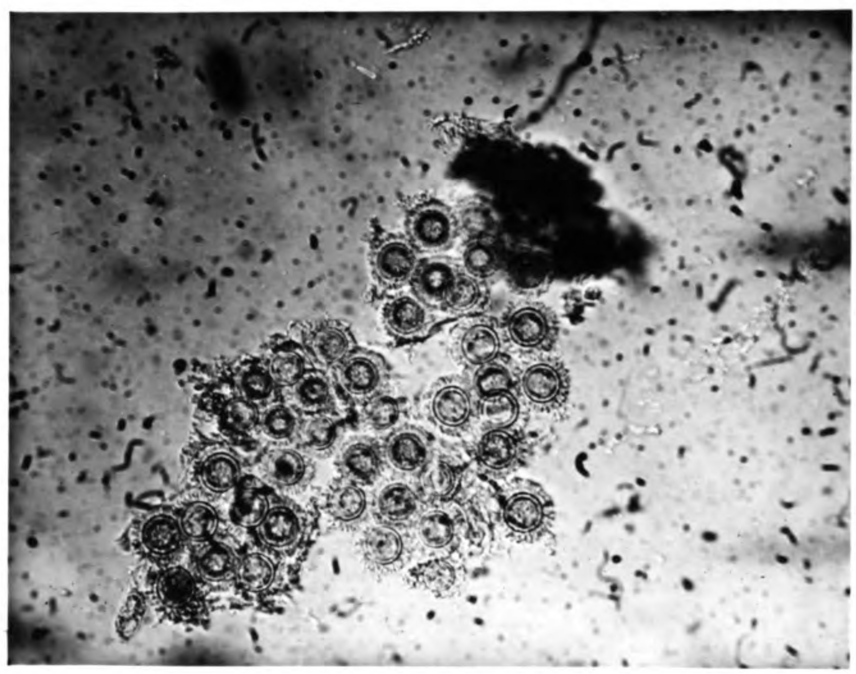


Figure 2. IMMATURE ASCOSPORES SHOWING ECHINULATIONS X225

PLATE IV

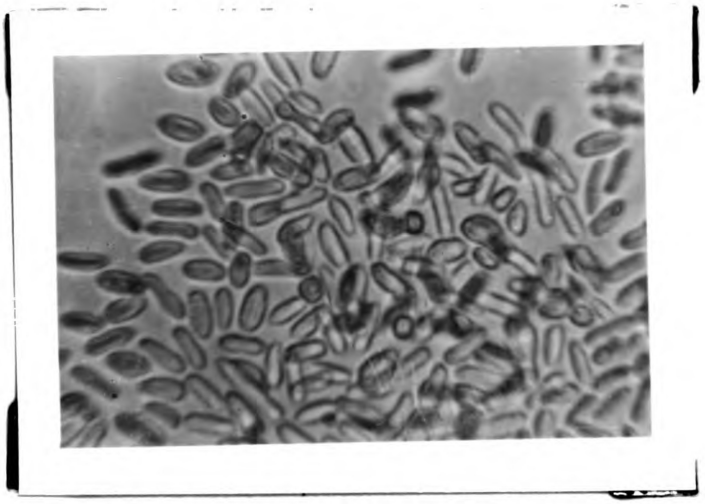


Figure 1. VARIOUS FORMS OF CONIDIA X500

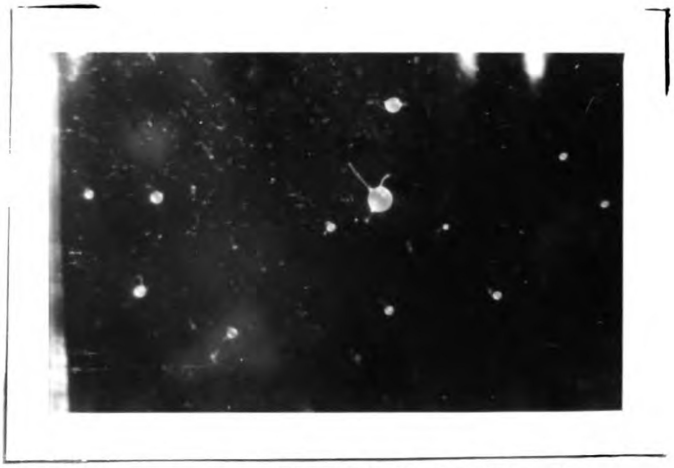


Figure 2. CONIDIAL HEAD X5

PLATE V

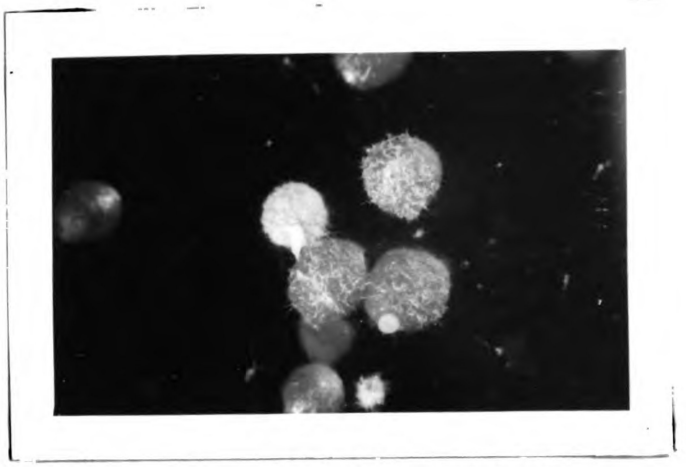


Figure 1. PERITHECIA X22

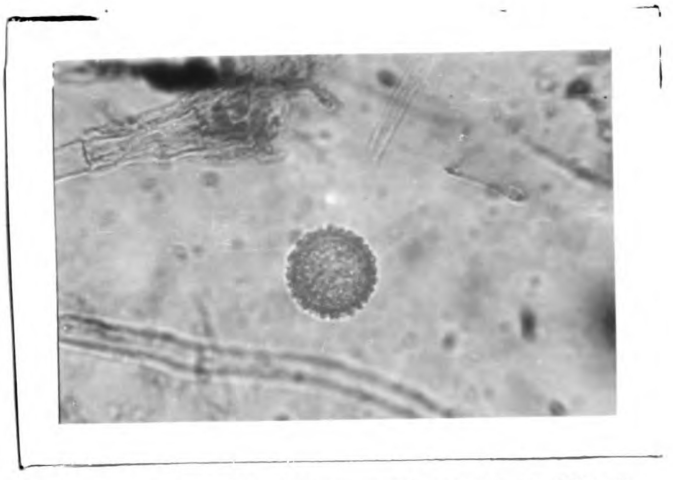


Figure 2. MATURE ASCOSPORE X500

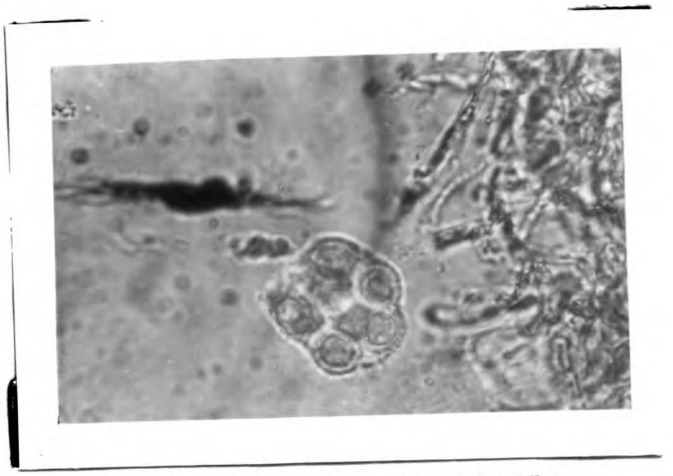


Figure 3. MATURE ASCUS X500

VI. SUMMARY AND CONCLUSIONS

1. This fungus was found in East Lansing, Michigan by Dr. Ernest Bessey, in the Fall of 1944 in some stable manure.

2. Single cell isolations of condida and ascospores were made and germinations of both types of spores produced an asexual stage belonging to the genus Gliocladium and a sexual stage of the genus Lilliputia.

3. A culture, resulting from a single ascospore germination, was grown in vitro and used to determine a) optimum temperature, b) suitable carbon and nitrogen sources, c) optimum hydrogen ion concentration, d) possible synthetic media which could be used to produce both stages of the fungus, e) growth on various organic media, f) the effect of light, intermittent light, and darkness on growth, conidial measurements, and fruitification of the fungus and, g) the combined effect of varied temperatures, pH's and media on growth, fruitification and morphology.

4. In general, organic media were quite suitable for growth and reproduction of L. margaritacea in that both stages were produced in abundance in a short time (10 days).

5. In an attempt to produce a synthetic medium on which the fungus produces both stages, varied molarities of sugars were used in combination with two nitrogen sources (KNO_3 and a-aspuragine). The combination of .03 molar maltose and a-asparagine resulted in conidial formation. No fruiting was found in other combinations.

6. Fries and wheat agars were used in an experiment showing the effects of temperature, media and pH on the growth and measurements of the fungus. The following conclusions were drawn from the results obtained;

Wheat agar

a) Variation in pH at a constant temperature causes appreciable differences in measurements of conidiophores, conidia, and metulae. Other structures are fairly constant.

Fries modified synthetic (plus .5% yeast extract);

a) Variation in pH at a constant temperature causes appreciable differences in measurements of conidiophores, branches, phialides and metulae.

7. Fungus growth occurred from 14-30°C, the optimum temperature being 24°C. The pH optimum differs with media used.

8. The effect of light on fruiting was influenced by the media employed. On most media, however, perithecia and conidia were produced in greater abundance in the absence of light.

9. No effect of light upon the production of the three types of conidia found could be ascertained.

10. Optimum pH and temperature for sporulation were determined to be as follows;

On wheat agar,

1. For conidial production- pH 3.0 at 28°C.
2. For perithecial production- pH 7.4 at 24°C.
3. For both conidial and perithecial production- pH 7.4 at 24°C.

On Fries agar

1. For conidial production- pH 7.4 at 30°C.
2. For perithecial production- pH 6.5 at 24°C.
3. For both conidial and perithecial production- pH 6.5 at 24°C.

11. Size of conidiophores, spores and metulae was appreciably

altered by a change in pH of wheat agar. Other characteristics were not affected.

12. Size of conidiophores, conidiophore branches, phialides, and metulae was affected by a change in pH of Fries synthetic (plus .5% yeast extract) medium. Other characteristics were not affected.

13. Size of conidiophores, branches, metulae, phialides, and perithecia was found to be affected by the media used.

14. The variability of the taxonomic characters of the fungus under consideration, under different environmental factors having been determined, a comparison of this fungus with the descriptions of Gliocladium prolificum, Lilliputia gaillardii, Lilliputia insignis,---three known species which bear close resemblance to our fungus---led to the conclusion that our fungus is a hitherto undescribed species for which the name Lilliputia margaritacea is proposed. A formal description is appended.

APPENDIX

Species of Lilliputia and Gliocladium Described in the Literature

Gliocladium penicillioides Corda. 1840. Icones Fungorum IV: 30. Pl. 7, fig. 92.

Matruchot. 1895. Revue Général de Botanique 7: 323.

Penicillus clusters very small, punctiform, white stipe erect, flexuous, wavy, thickened above, septate, powdery white, branches opposite, branchlets verticillate and in fours, crowded. Head of spores globose, white, spores conglutinate, oblong, bound by a thick gelatinous layer. Each spore has a thick gelatinous layer. Length of spore 5 μ.

Grows on the hymenium of decaying Thelephora hirsuta and Th. sanguinolenta.

The whole plant reaches 1/10-1/6 of a line (.1 inch). Clusters are visible with only a sharp eye as they are small white points. They rarely reach the diameter of 1/2mm.- every cluster consists of 15 or 20 conidiophores which grow upward and twine. They grow up winding together and later upright. Under low magnification, stalks appear like threads, club shaped at the top which bear spore heads. Under the high power, consists of branches and branchlets which have the spore head on top and the end branches producing the spores. The spores are very small, oblong, and somewhat unequal and obtuse. They are white and surrounded by a clear white thick slime layer. The stipe and its branches are divided and transparent-white, hollow with roughnesses externally. Branches and branchlets are attached just

below the septum. The thick branches are opposite - the thin branches and last branches are thin, whorled and in fours.

Gliocladium lignicolum Grove. 1886. New or noteworthy fungi. Jour. Bot. 24: 199.

A Gliocladium with gregarious or fasciculate hyphae, erect, equal thickness, 3-4 septate, hyaline, at apex penicillately ditrichotomous; head of conidiophores globose or obovate, white; conidia stuck together in mucous, hyaline, oblong-ovoid 2-2½ by 1½ μ.

Was found on the surface of wood Aug. to Sept. Does not differ from G. penicillioides except by the conidia being ½ as long, and its habitat. Perhaps it would be better to consider it a variety. Sometimes the hyphae in the lower dry part of the wood are 5 branched and colored brown.

Eurotium insigne Winter. 1887. Rabenhorst. Kryptogamenflora von Deutschland, Oesterreich und der Schweiz. Zweite Auflage 1 (2): 61.

Mycelium sparse. Perithecia superficial, round, waxy, yellow, soft, membranous, smooth - .25 -.4mm. Asci are irregular, oblong, containing 8 spores. Asci disintegrates quickly and are 35-45μ long by 28-30μ wide. Spores are pressed together, roundish, almost colorless and heavily covered with short spines 14-16μ in length. This culture was collected from goose manure from Holle.

The author mentions conidia present but no characteristics are given. Some possible relation to G. penicillioides is indicated.

Gliocladium conjunctum Cooke et Massee. 1887. Some Exotic Fungi. Grevillea. 16: 16.

Clusters minute, punctiform, rust color. Hyphae erect, crowded, forming compact clusters, septate, mostly simple; head of conidia cuneate, pale smoky, long, covered with mucous; conidia stuck together, oblong and in chains 5 by 3μ, smoky-hyaline.

Found on paper from India.

Matruchot. 1895. Rev. Gen. Bot. 7: 451.

In carrot, color varied from white to orange. Fertile branches short, slender, and approximately 200μ high, 3μ in diameter, hardly larger than the creeping mycelium. In the lower part of the culture tube, spore heads are so abundant that together they give the appearance of a uniform mucilaginous mass covering the substratum. In old cultures the orange color remains indefinitely.

Spores are 4-5 by 2-3μ.

Gliocladium agaricinum Cooke et Massee. 1889. New British fungi. Grevillea 17: 30.

Causing the pileus of mushrooms to crack into large frustular scales.

Tufts hemispherical, sometimes confluent, pallid, growing white at first gelatinous. Hyphae creeping, branched, fertile, erect branches producing ultimate verticillate quaternate, capitulum of conidia-subglobose, white, hyaline, 5-6μ in diameter.

Found on cultivated mushrooms, Leicester.

Gliocladium viride Matruchot. 1893. Bull. Soc. Myc. France 9: 249-252.

This species was found on Clitocybe; collected in a woods in

France in April. Appeared in the form of small green droplets of mucilaginous substance supported on short conidiophores.

Once collected, G. viride was grown on carrots, turnips, gelatin, etc. A germinated spore gives rather variable mycelium from 3-6μ; septate, branched, with swellings here and there.

The diameter of the conidiophore enlarges slowly up to 12μ and the end becomes spore bearing. Branches of several sizes 3-6μ to 2-3μ. At the end of each brush, there are first distributed oval spores arranged in chains and for so etime head like. These spores secrete sticky material that envelops the spores into a sperical green mass.

Branching of the conidiophore occurs producing new branches directed downward and completely giving a distinct root like or anchoring effect.

Gliocladium macropodinum E. Marchal. 1895. Champignons Coprophiles de Belgique. Bulletin de la Soc. Royale de Botanique de Belgique. 34: 135.

Clusters white, delicate, sterile hyphae slender, flexuous, creeping, fertile ones erect, strong, 3-4 septate, 350-400μ by 14-16μ bearing a mucilaginous white little head, the basidia several, conidia conglutinate, hyaline, 9-11μ by 2-2½μ, oblong, continuous with epispore smooth and guttulate.

On dung of kanguroo in Belgium.

Lilliputia gaillardii Boud. et Pat. 1900. Bull. Soc. Myc. France 145-146.

This small species is hardly visible to the naked eye. It is seen as small whitish grains which have not been seen with mycelium,

and are dispersed in the humus of used bark as those of Coccobotrys and Xylopilus were found. The author mentions its resemblance to Terfezia but is well distinguished not only because of a difference in width but also because of the composition of the peridium. The cortical layer reaches about the middle of the central portion and is 35-90μ thick. The cortical layer is made up of polygonal cells which are rather large in the central region, and smaller and more granular at the surface and near the gleba. It is a little more colored than the gleba which is entirely white and seems to be formed of jelly-like material in which the asci are oblong without the appearance of basilar enlargment which may be there and are 22-24μ in diameter which contains 8 spores, seldom less, and which resemble Terfzia in size and warty appearance. These ascospores seem to have oily drops and at maturity are light colored but visible.

The mature spores are light brown and look white when seen through the perithecium. As shown in plate V, asci which enclose mature ascospores are loose.

The perithecia are not over ½ mm.

Gliocladium hyponyces Sacc. 1907. Syll. 4: 80.

Same as Penicillium hyponyces.

Scattered white, hyphae; erect septate, hyaline, with erect apex, capitate. Di-to tri-chotomous, Conidia catenulate, ellipsoidal, hyaline 3-4 by 2μ On Stereum hirsutum in England. Close to P. candidum from which it seems to differ by its ellipsoidal conidia and its more regular branches.

Gliocladium roseum Bainier. 1907. Bul. Soc. Myc. France 23: 111-112

Plate 15. fig. 1-6.

Conidia: two conidial appearances-colorless to pink, in mass, 5-7μ by 3-5μ, slightly apiculate. On potato dextrose agar, colony is white to pink or salmon in fruiting areas which are produced at first, the hyphae being in ropes. Dense pinkish masses are produced.

Mycelium: branched and septate.

Conidiophores: consist of more or less elongated support arising from a single terminal verticil of 3-6 long sterigmata. Each sterigma produces a series of conidia coated with a gelatinous substance producing one spore after another. These conidia do not remain together on chains. Right after forming they slip successively on conidia underneath to which they stick forming a large spherical mass. Normal conidiophores are produced much later and soon cover the total surface of the mycelial mass. These conidia give a rose coloration. Conidiophes send out one or two lateral branches which in turn form 1 or three small branches which branch out again or terminate in 3 or 6 short sterigmata.

This fungus has the property of producing two fruiting systems. The first has already been discussed, and the second; long cylindrical branches of conidia which remain united, and lie side by side and parallel.

Gliocladium pulchellum Penzig et Saccardo. 1909. Syll. 18: 521.

Loosely gregarious, hyphae fertile erect, simple, continuous (not septate) white, 1 mm., tall, apex not inflated, base 40-50μ thick, Heads globose, hemispherical, dirty rose colored. Basidia

very dense, pencillately radiating, forked or simple, filiform, 30-90μ by 1.7-2μ, conglutinated by mucous; conidia minute, ovate-ellipsoidal, in chains, 2.3-3 by 1.7-2μ, pale.

Habitat; on thalli of lichens, on living shoots and leaves of mosses and probably parasitic. Tjibodas, Java- very distinct on account of non septate hyphae. Probably type of a new genus to be called Cladoglium.

Gliocladium luteolum Von Prof. Dr. Franz V. Hohnel. 1903. Mycologische Fragmente. Ann. Mycol., p. 523.

Sterile hyphae obsolete-fertile scattered, smooth, dilutely ochraceous, hyaline above and thinly tunicate about 6μ thick; below more thickly walled about 10μ thick-about 5 septate-360μ tall, penicillately branched above. The branches verticillate and these again branched. Crowded parallely spores on apical branches, conglutinate into a globose yellowish head 30-100μ thick-very numerous spores, hyaline oblong continuous hyphae 5-7 by 3μ. Collected scattered on rotten wood in forest at Wassergesprenge, Wienerwald, lower Austria in Oct. 1903.

Gliocladium nicotianae Oudemans. 1909. Saccardo 18: 521.

Sessile creeping hyphae, a little thickened at the base hyaline-fertile hyphae, erect septate, hyaline, branched. Primary branches quaternate-verticillately, crowded, cylindrical, 32-43μ long; secondary branchlets turnate, basidia arising 2 from each secondary branch, cylindrical, hyaline 16μ long. Conidia oblong, hyaline, continuous, 8-10μ by 3-4μ and conglutinate into a head forming a gelatinous droplet 1½mm. in diameter.

On decaying leaves of Nicotiana tabaci, Amerongen, Holland (collected by C. J. Koning).

Gliocladium elatum Sacc. 1909. Ann. Mycol. 7: 434.

Mycelium white, cottony, loose, shortly spread out. Creeping sterile hyphae few-conidiophores all erect, long, 1500 by 4-5μ filiform, septate, capitate at apex, heads globose ovate, interior mucous for a long while firmly conglutinate and under lens opaque, but soluble in glacial acetic acid. Pseudo-basidia twice verticillate, primary and secondary fasciculately turnate or quaternate. Conidia catenulate, small, subglobose, smooth 2.5-3 by 2.5μ hyaline.

In old wet partially decayed pilei of Schizophyllum commune in green houses of the Botanist garden at Padua, April 1909. Close to Glio. hypomyces but differs by its conidiophores 4-5 times longer although narrower, by its spherical conidia and by growing on Schizophyllum instead of Stereum.

Gliocladium prolificum Bainier. 1910. Bull. de Soc. Mycol. 26: 335-339. T. VI. Pl. XXI.

Found on straw.

Conidiophores; branching at practically every septum. Conidial apparatus consists of two layers of 3-6 verticillate branches which in turn consist of one or two cells. Each upper branch has 3-6 sterigmata-dilated at the base and becoming smaller at the tip. Length 8-9 times the largest diameter of the base.

Conidia; irregular and unequal in size, producing one below the other, some round but most of them oval; 4.2 by 6.3μ. A sown conidia produces a conidiophore apparatus, horizontal hyphae, conidiophores

with a diameter more than 9.6 μ and produces a quantity of perithecia.

Conidia formed, gelatify their most external membrane and slide one over the other to form a regular spherical mass of variable diameters. After a while depending on temperature (15 days to three weeks) a net work is formed from which is derived perithecia in a spherical mass of variable dimensions which can attain one mm. or more at maturity. Asci appear later containing 8 spores.

Perithecia; composed of large polyhedral cells of coffee with milk color. In the internal cavity, asci are borne on short filaments, in the form of an irregular, oval sac which disintegrates releasing spherical spores 25-28μ covered with a membrane ornamented with small protruberances which are isolated but many times united. The height of these protruberances measures 2μ. Spore color is yellow-brown. Upon germination mycelium is produced resembling that of conidia but does not germinate as fast. This was thought to belong to the Perisporiaceae.

Gliocladium piliforme (Pers) Boud. 1905-1910. Icones Mycologicae ou Iconographie des Champignons de France. E. Boudier. Tome IV Planche 537. 346-347.

This small species is composed of numerous filaments united in colonies, erect and ending at the peak by a small rounded white head formed by the united spores on the top of a small capitulum. These filaments are rigid but nevertheless are a little flexible, simple in all their length except at the extreme peak where they are divided in small penicilliform branches; they are septate, blackish when seen thru the magnifying glass but are rust under the microscope, divided

at the peak in 2 or 4 very short branches, evenly colored which support other uncolored (hyaline) cylindrical divisions; these are then divided at their peak in 4 others, these supporting elongated sterigmata which produce the conidiospores. The whole is held together by a water soluble mucilage in a globose head which seems supported at the base by the 2-4 small colored branches. The spores are hyaline, elliptical without granulations and are 3-9μ long by 3½-5μ wide.

This small species is found on rotten wood, where one does not see it except by the aid of a magnifying glass as small black bristles surmounted by a small white head. Examples shown have been recovered in February on the wood of a dead poplar.

Gliocladium deliquescens Pinkerton. 1936. Ann. Mo. Bot. Garden 23: 47.

Colony; at first colorless, of mainly submerged hyphae quickly becoming thin and white-floccose over the surface then meadow-green. Slimy on carrot plug and blackish green on P.D.A.

Conidiophores; two types- penicillate and simple cephalosporial becoming green and aggregate in a heavy slime, Triseriate and each series trichotomous; brush 45 by 40μ; first series 17-24 by 4μ; second 9-11 by 4μ; phialides 6.9 by 2-2.5μ, spores 3.5 by 2.5μ.

Sopp, Olav Johan-Olsen. 1912. Monographie der pilze-gruppe Penicilium. Videnskapselskapets Skrifter I. Mathematisk-natur-videnskabelig klasse 1912: 39-93.

When atmosphere is damp and the fungus well nourished, conidia flow together in ½ oily, ½ syrupy, difficultly flowing liquid which finally sinks whole fungus.

while attached to sterigmata the conidia are pointed at both ends but later are somewhat rounded.

Hab. Found growing on the upper side of Daedalea unicolor.

Thom C. & K. Raper. 1949. Manual of the Penicillia. Williams & Wilkins Co. pg. 630-631.

Penicillium-like conidiophore which later becomes enveloped in slime masses as the conidial chains dissolve and run together; reverse gray at first, later dark green, almost black, odor characteristic; gelatin liquified; conidiophores up to 1 mm. long, erect, course, septate, 1-5 times penicillate branching. Conidia about 1X1.5-2.0μ or somewhat larger when ripe, at first fusiform, later becoming more rounded at the ends, produced in chains which break up as the conidia become enveloped in masses of slime; perithecia and sclerotia not found.

Gliocladium africanum Eichelb. 1913. Saccardo 22: 1279.

Solitary, scattered, with sterile hyphae penetrating the substratum. Conidiophores erect 170-270-600 by 3-9μ. The apex not thickened, faintly yellowish, remotely septate, paler above, penicillately branched with lower whorls in 3's. Branches closely crosded, conidia acrogenous, solitary together with branches and branchlets surrounded with mucous, hyaline, smooth, ovoid 4 by 2μ.

Hab. On decaying wood, oblong with Tilmadoche nutans, Amani, East Africa.

Gliocladium cinereum Marchal. 1931. Contributions a l'étude des champignons fructicoles de Belgique. Bul. Soc. Roy. Bot. Belg. 54: 150.

Clusters dense, short, ashy with sterile hyphae creeping and

copiously septate, about 7μ thick. Conidiophores, 3-4 septate, erect or ascending, simple, 3-4 times verticillately branched. Producing a type of brush 40-60μ by 3½-5.7μ. Conidia globose, somewhat ovoid, one drop of oil in each conidium, 2.3-3.4μ in diameter surrounded by thick mucous.

Hab. On pear fruit (Gemblaux)

Observation- By its smaller spores and by its color, it is clearly distinct from other species.

This species was found on pear of the Beurre. It entirely covered the mummified fruit with its ash colored gauze. Colony chlamydospores 13 by 15.5μ, terminal 5 by 11μ; akinetes 15μ in diameter; arthrospores 10.5 by 5μ; oidia 3 by 5.5μ, rare.

Hab. Parasitic or semi-parasitic on certain species of Areca.

Gliocladium fimbriatum Gilman and Abbott. Oct. 1926-July 1927. Iowa State College Journal of Science 1 (3): 302, 304.

On Czapek's agar; colony is pure white at first with zones of dark green fruiting areas appearing at the center of the colony. Conidiophores arise from aerial hyphae, smooth and up to 25u long. Heads are enveloped in round balls of slime in which chains are not distinguishable. Fructification is in two stages, branches and metulae. Conidia may be born directly on phialides which arise directly from the conidiophore. In most heads branches arise some distance below the main head. Phialides 10-20μ long. Conidia elliptical or elongate, ovoid, smooth, pale green, 6.5 to 9.5μ by 2.5 to 4μ. "From soil: U. S: Iowa, Louisiana."

Gliocladium atrum Gilliman and Abbott. Oct. 1926-July 1927. Iowa State College Journal of Science. 1 (3): 302, 305.

Colonies on Czapek's agar: brown-green, small, slowly spreading, largely submerged, aerial mycelium olivaceous, scanty, aerial growth consisting mostly of conidiophores; colonies moist with slime which envelopes the heads. On bean agar considerable mycelium is produced. Conidiophores arise as olivaceous, thick walled, smooth, and septate, 75-300 by 3-4u. Fruitifications are in three stages, sometimes in two or four. Branches 3.5-9.5 by 3-3.5μ; metulae oblong, 7.5-9.5 by 3μ; phialides 7.5-10 by 1.5-2.5μ. Conidia oval to ovoid, 2.5-4μ by 2-2.5μ.

Hab. From soil. U. S. Louisiana.

Gliocladium catenulatum Gilliman and Abbott. Oct. 1926-July 1927. Iowa State College Journal of Science. 1 (3): 302, 303. Fig. 37.

On Czapek's agar; pure white becoming olive green to bright green in center as fruiting areas develop- fruiting areas were confined to the center of the colony. Concentric zones separated by sterile mycelium. Color reverses from colorless to yellowish. Aerial mycelium abundant, simple or in ropes from which conidiophores arise.

Conidiophores once or twice branched, coarse, rough, pitted, 50-125u long.

Fruitification in 3 stages; elements of fruitifications pitted or rough. Primary branches 15-20 by 3.5-4μ, metulae 7-9 by 15-25μ, phialides 10-20μ.

Conidia elliptical, smooth, pale green 4-7.5 by 3-4μ.

Isolated from soil.

Gliocladium microsporium Petch, T. 1926-27. Additions to Ceylon fungi. Ceylon Journal of Science. 10: 137.

White, forming sub-cylindric or spreading fascioles, up to .6mm. high, .1mm. diameter at the base; conidiophores branched near the base, simple above, 4u diameter below, slightly attenuated upwards, 3μ diameter above, hyaline, septate, straight, somewhat rigid, closely and minutely verrucose, bearing at the apex a few, usually 4, cylindrical prophialides, curved below, 8μ long, 2μ in diameter, each bearing two to four elongated flask shaped phialides, 8-16μ long, 1.5μ below, the prophialides and phialides forming a lanceolate head, 16-24 by 8-10μ; conidia adhering in a globose mass up to 30μ in diameter; conidia hyaline, oblong-oval, 1.5-3 by .75-1μ or subglobose, 1μ in diameter.

On Polystictus flabelli-formis.

Gliocladium flavum Van Beyma Thoe Kingma. 1933-30. Ueber zwei von Hevea-Rinde isolierten Pilze aus Sumatra, Mitteilung aus dem "Centraal Bureau voor Schimmelcultures". Verhandelingen der Koninklijke Akademie van te Wetenschappen, Amsterdam, (Twcede Sectie). 26.

Clusters on malt-beer agar dark yellow-woolly growing in zones and spreading rapidly- underside orange yellow. Hyphae-sterile-creeping, fertile rising- fertile 2-3 mm. high- hyaline septate- 4μ broad. Conidiophores not abundant, smooth, septate, irregular going off right and left from mycelium. 50-90μ long, 3-4μ broad- 1-3 branches- every branch at tip with 3-6 sterigmata. The latter are elongated, bottle shaped, 14-16μ long by 1-.7μ broad- narrow towards upper end. Many times the sterigmata are surrounded by slime

and form in this case spheres of 10-15μ in diameter out of which touching with glass needle, great masses of spores spread out. Conidia bean shaped to ellipsoid, smooth, hyaline, 4.3-9.7μ long (mostly 4.7-6.7μ mean of 100 spores 6.08μ) and 2.3-4.7μ broad. (Mostly 2.7-3.7μ mean of 100 spores 3.25μ). The spores germinate easily in water in the process of swelling to double their size.

Pure cultures:

On rice- after 7 days becomes sulfur yellow but at upper end of tube little white mycelium.

On Raulin agar- after 14 days, thin, slightly wooly skin, greenish white.

On Oat malt and Corn meal- after 14 days- almost nothing grown but on surface, numerous small drops of water- on corn meal a few small orange yellow colonies.

40% Saccarose- after 7 days, thin skin. The inoculated places are granular, whitish yellow and after 14 days orange-yellow.

Carrot- after 7 days strongly radiating mycelium, after 14 days becomes yellow but carrot not entirely covered.

Potato- in 14 days, white wooly mycelium, but pieces not entirely covered.

Found on bark of Hevea (India Rubber tree) Sumatra.

Gliocladium nigro-virescens Van Beyma Thoe Kingma. 1931. Ueber ein neues Gliocladium. Mitteilungen aus dem "Centraal Bureau voor Schimmelcultures". III. Verhandelingen der Koninklijke Akademie Van Wetenschappen te Amsterdam. Tweede Sectie. Deel 29: No. 2: 30-32, fig. 1.

Mycelium on beer-wort agar in a petri dish not vigorous, sane-

what crisp woolly. Mostly consisting of bundles of hyphae at first greyish white then light green about 347 in Klincksieck and Valette Code of Colors: growing in several zones which after several weeks become darker by conidial formation (342-373. K & V). At a greater distance from the point of inoculation, the mycelium is always more sparse but on the contrary the masses of conidia are more numerous so that the outer zones consist only of conidiophores. The marginal zone is formed of mycelium lying flat on the agar, grey or somewhat reddish (23A), the underside of the petri dish is yellow (228A-211-206). No typical odor. Under the microscope, mycelium shows numerous oil droplets. Conidiophores branched or unbranched, smooth, 40-100μ long by 3-4μ, greenish with numerous oil droplets. Metulae 15-25 by 2.7μ.

Phialides- flask shaped often with long neck, 20-25 by 3.7μ.

Conidia- oblong or ellipsoidal, often somewhat pointed at one end. Sometimes bean shaped or more rounded. Smooth, greenish, singly, in mass: black-green with 1 or 2 indistinctly visible darker spots in interior. United in great masses mostly in slime heads 15-70μ in diameter.

In pure culture; after 7 days at room temperature

Beer Malt agar; little loose woolly white mycelium over a greyish black agar surface due to conidial formation. 250-275. Reverse yellow 211.

Carrot- no aerial mycelium. Conidiophore head covered with conidia for most part sparsely, but in larger numbers on the sides. Dark green.

Potato- overgrown with vigorous developed but little aerial mycelium. Violet above (503A) then follows a zone with many conidia

375 while lower down fungus grows leathery.

Hab. In garden soil Baarn, Holland.

Gliocladium Vermoesani (Biourge). Pinkerton. 1936. Annals of the Missouri Botanical Garden. 23: 47-48.

Colony: pale v[illegible]nuc[illegible]ous, growth rapid producing much guttation water.

Conidiophores: mostly penicillate measuring 45μ from the first branch, asymmetric, polyseriate with the phialides trichotomous and measuring up to 10 by 1.5-3.5μ, spherical spores 2.5μ; cephalolides 40μ and more, cephalosporia 20-25μ, cephalospores ellipsoid 5-6.6 by 4μ; intercalary chlamydospores 13 by 15.5μ, terminal 5 by 11μ; akinetes 15μ in diameter; arthrospores 10.5 by 5μ; oidia 3 by 5.5μ, rare.

Habitat; parasitic or semi-parasitic on certain species of Areca (palms).

Thom, C. & K. Roper. 1949. Manual of the Penicillia. pg. 630-631. Williams & Wilkins Co.

Colonies on wort gelatine, producing salmon colored coremia 10 mm. in height or more; conidiophores about 5μ in diameter; metulae 7-15μ by 2.5-5.0μ, irregularly borne, irregular in number or none; sterigmata 10-20μ by 2.5-3.5μ in groups of 2-5, or even 7; conidia elliptical 5.0-7.5μ by 3-4μ.

Gliocladium album Petch. 1937-38. British Mycological Society 22: 261.

Petch writes of a Gliocladium occurring on Myxomycetes and had been recorded as Gliocladium penicillioides. A white crust is formed over the sporangium, the main stem being 5μ in diameter with lateral

branches beginning at the first septum about 20μ from the base. Branches are opposite or in whorls of 3 and divergent. These branches again branch and the conidiophores interlace. The phialides are 13 by 1μ. The stem and branches are minutely verrucose and larger at the nodes. The conidia are oval or oblong-oval, hyaline, 2.5-4 by 1.5μ. This is thought by Petch to be the same as Penicillium album Preuss and Petch gives it the name Glio. album (Preuss) Petch.

Gliocladium strictum Petch. 1938. British Hypocreales. Trans. Brit. Myc. Soc. 21:

Conidial stage- conidiophores crowded, white in mass, hyaline, 100μ high, stem stout (4μ diameter below), few branches, lower branches solitary and distant, upper branches phialides opposite or in whorls of three, about 30μ long, tapering, all branches parallel to main stem. Conidia hyaline, oblong-oval or narrow oval, slightly inequilateral, ends obtuse, 5-11 by 2-2.5μ, the longer ultimately becoming one septate or pseudo-septate, united by mucous in heads about 14μ in diameter, and in large masses.

Sexual stage; Hypomyces Broomeanus Tul., Perithecia ovate, .25-.3mm in diameter, hyaline becoming pale brown, densely clothed in white or pale brown short tomentum, except at apex, superficial or immersed through overgrowth of the tomentum; asci cylindrical 130-140 by 4μ; ascospores fusoid, 1 septate, coarsely worted, shortly apiculate at each end, hyaline, 13-16 by 3.5-4μ.

Gliocladium caespitosum Petch. 1938-39. Gliocladium. Trans. Brit. Myc. Soc. 22: 257-268.

Conidiophores forming white or yellow tufts, devaricate up to

150μ high. Below 5-6μ in diameter. Smooth, branched above, the lower branches solitary, upper opposite each branch bearing a few apical phialides which are narrowly ampulliaceous phialides 20μ high and 2μ diameter. Conidia hyaline, oblong oval or narrowly oval sometimes uniquslateral, sometimes slightly curved, smooth, 5-8 by 2-3μ.

On immature Nectria mammoidea Plow.

Gliocladium municola Wei. sp. nov. 1941. Sinensia 12: Nos. 1-6. 140-141.

"In fructibus Pruni mume, Szechuan, China.

On rotten fruit, conidiophores appear as sparse downy growth on the surface, erect, simple, occasionally branched, septate, hyaline or brown and guttulate at the base, smooth, 188.8-302.4 by 7.7-12.1μ (excluding the penicillus); penicillus consisting of one series of metulae bearing a whorl of two to four sterigmata with the extension of the central axis as the central metula, 23.8-44.8μ high; metulae cylindrical, tapering toward the base and rounded at the apex, 39.2-67.2 by 2.5-3.0μ except the central metulae which may be more than 10μ in diameter; sterigmata 2-5, usually on a metula, acicular in shape, rounded at the base and tapering toward the tip, slightly curved, toward the axis of the metula, 11.2-16.8 by 1.4-2.0μ; conidia in chains then forming a head with mucilaginous fluid (43.7-123.8μ in diameter), elliptical to narrowly ovate or elongated elliptic, hyaline, biguttulate, very variable in size, 5.6-14 by 2.8-5.6μ.

On agar medium it produces a thin layer of white down of fruiting structure, later snuff brown and the reverse cinnamon brown to clove brown; aerial mycelium at first none, then loose and sparse; vegetative mycelium slender, 2.8-4.2μ in diameter and the fertile mycelium much thicker and sometimes tinted with brown, 3.0-9.8μ in .

diameter; conidiophores essentially the same as that on rotten fruits, arising perpendicular from the cells of fertile hyphae, 67.2-332.4 by 6.2-12.9μ; metulae of one to two series, or sometimes none, 11.2-23.5 by 2.4-7.0μ; sterigmata forming a verticil on the conidiophore or in a group of two to several on a metula 7-14 by 2-3μ; spores similar to those on the rotten fruits; submerged growth consisting of hyaline mycelium and brown chlamydospores; chlamydospores abundant on old cultures, forming irregular balls of 4-12 cells, 46.2-61.2 by 3.9-5.7μ; single cells globose to subglobose or irregular as the result of crowding, thick walled, containing numerous large oil globules, 12.6-29.4μ across.

The present species shows affinity for G. roseum (Link?) Bainier from which it differs by (1) the absence of loose floccose type of growth, (2) larger conidia and (3) the presence of chlamydospores.

On fruit of Prunus mume it produces a rot cinnamon buff in color, soft, with distinct and entire margin, then covered with whitish downy aerial growth, moldy smell distinct; infected flesh honey yellow in color,"

Gliocladium nigrum Moreau. 1941. Rev. Mycol. N. S. 5-6: 81.

Under Tortula and Lavende between Sainte- Marguerite and Formichet, France.

Aerial mycelium white, abundant on rich media (Malt agar)- only slightly developed on poor media. Cultures are radiating, at first colorless and then with black zones by the formation of conidiophores and conidia which macroscopically appear in mass sooty black. Conidiophores typical of Gliocladium, several times branched, the phialides elongated more or less cylindrical about 15μ long.

The conidia occur in chains, cylindrical, rounded at both ends, olivaceous, guttulate. Length 6µ-8µ, often 7µ, Width 2-2½ and exceptionally 3µ. Soon little chains agglutinate in spheres 10-30µ in diam; olivaceous-black which may flow together forming black shining patches. This species reminds one of G. viride Matruchot. But is distinguished by size and form of conidia which are black in mass- nigrum indicates this last character. This gives to the cultures the general aspect of the culture of Dematiaceae but all other characteristics make our fungus a true Gliocladium.

Gliocladium solani (Hartung) Petch. 1943-45. Additional notes on British Hypocreales. Trans. Brit. Myc. Soc. 26-27: 149.

This is a transformation and combination by Petch of Nectria solani Reinke & Berth, as the sexual stage and Spicaria Solani de Bary as the Gliocladium stage.

The perithecia are described as being crowded on an erumpent fleshy stroma; no definite cortex, forming pseudo-parenchymatous tissue without air spaces. However when Schroeter grew it in culture he found the hyphae did not form pseudoparenchymatous tissue. These masses are soft, yellowish internally, dark red above, and blackish externally.

The perithecia are reddish or dull yellow and become almost white, globose, .3-.4mm. in diameter with conical ostiolum. The asci are narrow clavate, with truncate apex, 65-72 by 6-8µ, the spores are uniseriate or biseriate, uniseriate below. The ascospores are oval, narrow, hyaline, becoming somewhat rough 10-13 by 4-5µ.

Conidiophores; scattered or gregarious. Stems 3µ in diameter, smooth, septate, branching below and bearing suberect lateral

branches. Conidiophores are in whorls at the nodes and in clusters at the apex, all attaining the same height.

Conidia; oval, 5-6 by 3-4μ, some 3μ and globose, hyaline, smooth remaining in a globose or irregular mass supported by the phialides.

Gliocladium cibotii (n.s.) Van Beyma Thos Kingma. 1944-45. Antonie Van Leeuwenhoek Journal of Microbiology and Serology. 10 (1-2): 46-48.

Hyphae- delicate, 2-3μ thick, light colored, hyphae go together in bundles which then exhibit a green brown to brown black color.

Conidiophores- go off from the bundles of hyphae in great numbers, 80-100 by 2-3μ, few branched, many forked with 2-3 sterigmata.

Sterigmata- straight, forming awl-shape, 20-40μ long and the base 2μ wide, mostly 2-3 verticillate.

Conidia- numerous, clusters glued together, hyaline, single celled, ellipsoidal 4-6 by 2-2.7μ mostly 4-4.7 by 2.3-2.7μ with 2 oil droplets.

Habitat- isolated from the hairs of the tree fern Cibotium Schiedei Frl. M. F. Habekotte, Delft.

Pure Culture;

In Beer Wort agar- after one month, colony 7 cm. in diameter, and in the center several white bundles of hyphae 1 cm. high. In the center a green-black felt like wrinkled mat with luxurient conidial formation. The edge consists of a zone of thin, colored, sterile branches of hyphae 1 cm. high having a delicate spicy odor; the underside of the center is black, in other places pale yellow.

In test tubes to 14 days

Beer wort agar- at the bottom of the test tube, white felt like mat with sparse conidial development.

Cherry agar- agar surface covered with small numerous whitish spore droplets. Here hyphal bundles are raised, 3mm. high, in cylindrical hyphal bundles which produce numerous conidiophores.

Carrot- covered with white felt like bundles of raised hyphae.

Whole potato- same as cherry agar- conidia numerous.

Oat agar- as above except at the edge near the glass, a black edge is produced, in potato agar, absent.

Lilliputia insignis (Winter) n. comb. Dennis and Wakefield. 1945-46. New or interesting fungi. Trans. Brit. Myc. Soc. 29.

On 29 March 1933 specimens of a fungus on mushroom compost from Uxbridge, Middlesex, were received at Kew from Mr. W. Buddin. The fructifications were spherical, glabrous, whitish to cream colored, approximately 500μ in diam. and scattered singly over the surface of the compost. Microtome sections showed them to have a stout wall, some 80 to 90μ thick, composed of ten to twelve layers of cells, differentiated into an outer belt, three to four cells wide, of empty cells, and an inner zone, 7-8 cells wide, densely packed with oil globules. The center of the ascocarp consisted of thin walled tissue in which were imbedded numerous asci, each with 8 spherical yellow ascospores. The latter measured 11-13μ in diameter with a large central oil globule and a wall about 1μ thick, densely studded with short blunt warts. These measurements were made on spores within asci.

Except for spore size, this agrees precisely with the diagnosis of L. guillardii described from humus in a glass house in Angers, France, April 1900. According to Boudier and Patouillard (1900) this fungus had spores 22-24μ in diam. Type material of the species was preserved by Pat. and is now in the Farlow Herbarium. Dr. Linder re-examined the material and reported as follows. He found the spores measured 13.2-13.2 by 13.2-24.5μ. These spores were for the most part still within the ascus and were somewhat compressed and angular. In the few cases were the spores had been released from the ascus, the spores are perfect globose. It appears therefore that the spores of this fungus vary greatly in size and that the Angers and Uxbridge collections represent the same species. It is also the same as the fungus distributed under the name G. penicillioides Corda as number 150 of Reliquiae Farlowianae. This had been collected on decaying seaweeds of Mittery Pt. Maine, U. S. A., on 5 July 1913 by Thaxter, who cited as synonyms P. insigne Bainier, Lipenicillium insigne Brefeld and L. guillardii Boud. and Pat. (Thaxter, 1922). Thom (1930), however regarded P. insigne as probably distinct from G. penicillioides and the applicability of the latter name is also dubious as will be shown below.

Previously Winter (1873) had obtained an ascomycete on goose dung which he regarded as the perfect stage of G. penicillioides. No description was published but material with adequate figures of asci and ascospores was distributed by Rabenhorst's Fungi Europaei, no. 1732, under the name Eurotium insigne G. Winter n. sp. (an n. g.?).

Examination of Winter's material at Kew shows it to correspond closely with the present fungus except that the ascocarps are now a

deep yellow brown and the outermost cells appear deep yellow in section. The ascospores measure 13-15 μ across. Massee and Salmon (1901), who obtained E. insigne on dung of kangaroo, Ovis burrhel, fowl, and horse, at Kew described it as having the perithecia clear white when young, then pallid, becoming yellowish and finally rusty brown, with ascospores globose, 17-20 μ across. The color difference mentioned above is thus clearly not significant and depends probably on the mode of preservation. Winter's collection has been dried for 70 years, the Uxbridge material has been kept in formalin.

These and other records of E. insigne have been discussed by Petch (1939) who concluded that the imperfect fungus associated with it is not G. penicillioides, which he ascribes to Hyponyces aureo-nitens Tul., but G. macropodinum Marchal. The name adopted by Thaxter is thus inappropriate both on the ground of mis-identification and of applicability only to the imperfect stage. Though association with a Sphaeriodium was not noted in the Uxbridge material nor mentioned by Boud. and Pat., it appears at present highly probable that all the collections cited above are the same species. Owing to the very characteristic and distinctive ascospores it seems inadvisable to refer this to Fungus to Eurotium; it would appear preferable to retain the genus Lilliputia, with the new combination L. insigne. Boud. and Pat. ascribed Lilliputia to the Tuberaceae, which is clearly inadmissable: Clements and Shear (1931) transferred it to Gymnoascaceae, in which it is clearly out of place. Pending detailed studies of the developing ascocarp it seems to fall most naturally in Eurotiaceae, near Eurotium, from which it is distinguished by its spherical warted ascospores.

Gliocladium aureum Rader. 1948. Phytopath. 38: 450.

Colonies on potato dextrose agar or Czapek's- at first pure white later yellow and occasionally light salmon pink in fruiting areas on Czapek's medium, mycelium loose-floccose, simple hyphae or hyphae bunched in ropes; sclerotia not observed; conidiophores perpendicular to medium, 40-180μ; penicillia up to 180μ in length, once or twice irregularly branched.

Phialides 10-15 by 2-3μ bearing conidia which may be aggregated in gelatinous masses or balls (Acrostalagmus type) or agglutinated in typical columns (resembling Clonostachys type), conidia hyaline, elliptical, apiculate, smooth, 3.3 by 5.9μ extremes (2.6-3.6 by 5.1-6.7μ).

A slight to marked yellowing of the medium in P.D.A.

Rab. Pathogenic on stored carrot roots in N. Y.

No data on life history.

Optimum Temp. 21-24°C- minimum below -3°C. Maximum between 33 and 36°C.

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