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Studies on *Diplodia* and *Diplodia*-like Fungi

I. Effects of Carbon Sources on Certain Taxonomic Characters and on Growth in Agar Culture

M. M. Satour, R. K. Webster, and W. B. Hewitt

II. Effects of Nitrogen Sources on Growth, Sporulation, and Certain Taxonomic Characters

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III. Variation in *Diplodia natalensis* from Grape in California

R. K. Webster, W. B. Hewitt, and F. J. Polach

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I. Effects of Carbon Sources on Certain Taxonomic Characters and on Growth in Agar Culture

Isolates of *Diplodia macrospora, D. natalensis, D. zeae, Botryodiplodia hypodermia, B. theobromae, Physalospora rhodina, Botryosphaeria ribis,* and a *Sphaeropsis* sp. were grown on synthetic agar media supplemented with 23 different carbon sources used either singly or in combination: L-arabinose, D-ribose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, L-sorbose, cellobiose, lactose, maltose, sucrose, cellulose, inulin, starch, xylan, raffinose, rhamnose, salicin, D-sorbitol, linolenic acid, palmitic acid, and pectin. Taxonomic criteria currently used to delimit these species—mycelial growth and color, stromata, pycnidial size and orientation with respect to the substrate, presence of septa, and morphology and exudation of pycnidiospores—differed, in most of the isolates, with the carbon source tested. For example, sorbose retarded mycelial growth and pigmentation but increased pycnidial production. Species on salicin developed pycnidia but not pycnidiospores. Inulin, alone or in combination with glucose, retarded hyphal pigmentation, the formation of pycnidia, and the maturation of spores. The effect of salicin was partially counteracted when it was combined with sorbose, glucose, or inulin. These results indicate the value and need for additional studies to establish standard culture conditions for use in taxonomic considerations of these fungi.

II. Effects of Nitrogen Sources on Growth, Sporulation, and Certain Taxonomic Characters

Twenty-eight nitrogen sources (20 amino acids, 2 amide derivatives of amino acids, 4 organic nitrogen, and 2 inorganic nitrogen) were used for culture of six isolates of *Diplodia natalensis* and one

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II. Effects of Nitrogen Sources on Growth, Sporulation, and Certain Taxonomic Characters[1]

ABSTRACT

Twenty-eight nitrogen sources (20 amino acids, 2 amide derivatives of amino acids, 4 organic nitrogen, and 2 inorganic nitrogen) were used for culture of six isolates of *Diplodia natalensis* and one isolate each of *D. zeae*, *D. macrospora*, *Botryodiplodia theobromae*, *Botryosphaeria ribis*, *Physalospora rhodina*, and a *Sphaeropsis* sp. Isolates were grown in synthetic liquid media and on synthetic agar media, supplemented singly with the different sources of nitrogen. Nitrogen compounds influenced mycelial growth and pigmentation, pycnidial size and orientation with respect to the substrate, presence of hairs on pycnidia, morphology of pycnidia and stromata and pycnidiospores, and exudation of the pycnidiospores. Results indicate that *Diplodia* and other related genera of fungi may use a wide diversity of nitrogen sources, but that the source may alter the taxonomic characters currently used to delimit this group of fungi.

INTRODUCTION

THE FIRST PAPER in this series showed that certain carbon sources influenced mycelial growth and formation of pycnidia and pycnidiospores, and altered most of the morphological characters of all species tested. This second paper reports results of similar studies with nitrogen sources.

With respect to nitrogen nutrition, Brown (1957) reported that mycelial growth of *Botryosphaeria ribis* Gross. & Dug. was maximum when glycine and asparagine were supplied in a liquid medium. Potassium nitrate, ammonium sulfate, and ammonium nitrate supported good growth, and sodium nitrite retarded growth. Brown accounted for the fact that the fungus made some growth in the basal medium without added nitrogen by indicating that some nitrogen was transferred with the inoculum.

According to Drake and Moore (1967), isolates of *B. ribis* grew moderately well to well on three organic nitrogen salts and on 15 different amino acids known to be commonly found in apple tissue.

MATERIALS AND METHODS

The species studied, and their isolate numbers were the same as those in the first paper: *Diplodia zeae* (Schw.) Lev. 130; *D. macrospora* Earle 35; *D. natalensis* P. Evans 6, 107, 147, 157, 213, and 230; *Diplodia* sp. 19; *Botryodiplodia*

[1] Submitted for publication March 27, 1968.

theobromae Pat. 29 and 44; *Botryosphaeria ribis* Gross. & Dug. 55; *Physalospora rhodina* (Berk. & Curt.) Cooke 86; and *Sphaeropsis* sp. 218.

The basal liquid medium (BLM) had the same chemical composition as BAM-A but without the agar. Portions of approximately 20 ml were put into

The basal agar media (BAM) used were:

COMPOSITION	BAM-A *gm/l*	BAM-B *gm/l*
Glucose	20.0	20.0
Potassium phosphate (monobasic)	0.0	2.0
Potassium phosphate (dibasic)	1.0	0.5
Potassium chloride	0.5	0.5
Magnesium sulfate	0.5	0.5
Ferrous sulfate	0.001	0.001
Biotin	0.0	4.0 (ppm)
Bacto agar	15.0	15.0
Glass-distilled water to make 1 liter		
pH after autoclaving	5.5	6.0

125-ml flasks. The pH of both BLM and BAM-A was adjusted with hydrochloric acid and potassium hydroxide before autoclaving. Plates containing BAM-A were incubated at 24°C and exposed for 8 to 9 hours daily to a fluorescent light (daylight type) of approximately 60 ft-c.

Early in the studies, we observed that light stimulated formation of pycnidia. Consequently, in experiments with BAM-B the cultures were exposed to a continuous light (Gro-Lux type) of approximately 250 ft-c for the 30 days of experimentation, after which, observations were made.

To avoid the transfer of some nitrogen with the inoculum, we grew cultures on a synthetic agar medium without nitrogen. All fungi grew satisfactorily on the BAM-A, to which they were transferred twice before being used in the experiment. The inoculum was a small disc (3 mm) of the fungus growing on BAM-A.

Nitrogen sources

The nitrogen sources were: DL-alanine, L-cysteine, L-cystine, glycine (A grade), L-isoleucine, D-leucine, DL-methionine, DL-serine, D-threonine, D-valine, L-valine, DL-aspartic acid, DL-glutamic acid, L-arginine, DL-histidine, L-lysine, D-phenylalanine, DL-tyrosine, L-hydroxyproline, L-proline, D-tryptophane, L-asparagine, DL-glutamine, casein hydrolysate, egg albumen, gelatin, protease peptone, potassium nitrate, and potassium nitrite.

The chemicals were analytical reagent grade to meet American Chemical Society standards. Chemicals were used at 1 gm per liter except for the last six nitrogen sources, which were used at a rate of 2 gm per liter. All nitrogen sources were added before autoclaving.

The entire culture on a given plate was added to 80 ml of water and mixed for 3 to 5 minutes in a Waring Blendor. Final volume was then adjusted to 100 ml. The pycnidiospores were counted in a hemacytometer.

TABLE 1

MYCELIAL GROWTH OF 10 ISOLATES OF *DIPLODIA* AND RELATED GENERA IN LIQUID MEDIUM A SUPPLEMENTED WITH VARIOUS AMINO ACIDS AS NITROGEN SOURCES

(Cultures incubated at 24°C for 30 days)

Nitrogen source	Mycelial growth (dry wt., av. mg/day)									
	Species and isolate number									
	Botryo-diplodia theobro-mae 44	*otryo-sphaeria ribis* 55	*Diplodia natalensis*					*D. zeae* 130	*Physalo-spora rhodina* 86	*Sphaer-opsis* sp. 218
			6	107	157	213	230			
Liquid medium A (control)*..........	8.56	5.00	6.14	4.36	15.22	8.44	9.78	3.14	12.11	2.93
Liquid medium A plus:										
L-arginine............	20.89	13.07	13.86	9.79	19.44	15.56	24.56	3.43	20.00	12.57
DL-asparagine.......	18.78	13.71	13.43	8.43	19.78	14.78	23.67	7.21	21.22	14.71
L-cysteine............	14.33	9.50	10.93	10.14	13.11	9.33	8.79	3.71	12.56	4.71
Glycine..............	13.89	14.71	17.86	11.29	18.89	10.89	16.33	3.00	15.89	13.21
L-histidine...........	21.00	17.50	17.00	10.79	25.22	15.67	20.22	5.43	24.44	13.93
DL-leucine...........	22.33	21.93	16.14	12.85	21.44	18.00	19.11	4.64	17.22	12.14
L-phenylalanine.....	11.22	12.71	14.00	9.29	18.67	13.00	23.00	4.71	13.78	13.00
DL-serine............	17.22	15.50	15.57	10.43	21.33	12.89	25.22	5.79	20.56	12.71
L-valine..............	14.33	13.00	16.93	10.64	18.22	10.00	28.79	3.21	15.44	14.79

* No nitrogen added.

RESULTS

Vegetative growth

In a liquid medium. On a dry-weight basis, the nitrogen source affected the mycelial growth of the 10 isolates grown in basal liquid media (BLM-A) alone or with any one of the nine amino acids (table 1). All 10 isolates grew very well on BLM-A. These fungi apparently utilize atmospheric nitrogen, the only source available in this experiment. Isolates of *Diplodia natalensis* 157 and *Physalospora rhodina* 86 grew more readily on BLM-A than did the other isolates, apparently being better able to utilize atmospheric nitrogen. Growth on the basal medium varied as much as threefold among the isolates. Also, the same isolate grew more on certain amino acids than on others. *D. zeae* 130 did not grow as well on the amino acids as did the other isolates. Growth of 130 was no greater on L-arginine, glycine, and L-valine than on BLM-A alone, whereas the other isolates, with few exceptions, produced more mycelium on BLM-A plus the amino acid than on BLM-A alone. Isolates of *D. natalensis* 6, 157, 213, and 230 grew unequally on BLM-A. Isolates 213 and 230, which grew about the same amount of mycelium on BLM-A, grew unequally on some of the same amino acids. For example, on DL-serine and L-valine, 213 produced only about half the weight of mycelium that 230 did. Isolate 230 grew a little less mycelium on L-cysteine than on BLM-A alone, but more than twice as much on most of the other amino acids.

On agar medium. Ten species of *Diplodia* and *Diplodia*-like fungi were grown on basal agar medium A (BAM-A), and seven on BAM-B alone and supplemented singly with 25 or more

TABLE 2

AVERAGE COLONY DIAMETERS (LINEAR GROWTH) OF ISOLATES OF *DIPLODIA* AND RELATED GENERA ON AGAR MEDIUM A SUPPLEMENTED WITH VARIOUS NITROGEN SOURCES

(Cultures incubated at 24 ± 2°C for three days)

Nitrogen source	Colony diameters (mm)									
	Species and isolate number									
	Botryodiplodia theobromae 44	*Botryosphaeria ribis* 55	*Diplodia natalensis*					*D. zeae* 130	*Physalospora rhodina* 86	*Sphaeropsis* sp. 218
			6	107	157	213	230			
Medium A (control)	72	46	45	44	76	80	70	14	72	17
Medium A plus:										
DL-alanine	32	28	35	35	44	35	42	0	49	42
L-arginine	65	46	38	40	76	74	74	0	72	0
L-asparagine	75	48	42	45	75	78	73	0	80	0
DL-aspartic acid	65	63	51	51	75	69	80	0	77	38
L-cysteine	77	32	42	41	75	80	60	0	78	0
DL-glutamic acid	52	38	48	44	61	53	66	0	59	77
Glycine	80	46	41	32	69	82	66	0	67	26
DL-histidine	55	52	41	39	80	78	77	0	72	53
D-leucine	68	45	49	45	74	42	55	0	72	0
L-lysine	61	53	37	42	55	64	60	0	62	4
DL-methionine	59	46	39	37	63	59	55	0	60	22
D-phenylalanine	59	45	32	45	64	75	65	0	67	0
L-proline	71	52	46	45	73	72	80	0	76	61
DL-serine	42	35	28	25	49	28	25	0	60	22
D-threonine	66	42	36	36	70	75	53	0	71	10
D-tryptophane	63	38	38	36	56	61	66	0	62	0
DL-tyrosine	65	50	53	44	80	75	77	0	75	25
L-valine	73	49	39	107	80	81	70	28	75	46
Casein hydrolysate	90	64	52	56	90	84	75	18	72	69
Egg albumen	82	71	60	55	88	81	72	46	85	73
Gelatin	79	48	52	58	83	74	75	12	71	66
Protease peptone	89	59	59	55	90	89	84	45	89	73
Potassium nitrate	79	53	60	60	85	70	75	21	68	67
Potassium nitrite	12	0	2	0	0	9	12	0	4	0

various nitrogen-source compounds. Growth was determined by averaging colony diameter on three culture plates at three days at 24°C.

On BAM-A (which had no biotin) all isolates started growth early and continued to grow well over the 30-day period (table 2), and all isolates except *D. zeae* 130 and *Sphaeropsis* sp. 218 grew on the BAM-A plus each amino acid or other nitrogen compound, except potassium nitrite. Although isolate 130 produced 14 mm of growth on BAM-A, it did not grow until after the fourth day on 18 (mostly amino acids) of the 25 nitrogen-containing compounds (table 2). Over the 30 days, however, it did grow on all of the nitrogen sources except potassium nitrite. On BAM-B (with biotin), isolate 130 started growth early on all of the same compounds except the following: D-leucine, DL-glutamic acid, L-lysine, DL-tyrosine, and D-tryptophane (table 3). Thus, biotin overcame the effects of delayed growth on 13 of the 18 compounds. Over the 30-day period, however, growth on the other five compounds was also very good.

On BAM-A, the initial growth of all isolates was slower on DL-alanine than on BAM-A alone (table 2). An isolate of *Sphaeropsis* sp. 218 was late to start growth on L-arginine, L-asparagine, L-cysteine, D-leucine, DL-lysine, D-phenylalanine, and D-tryptophane.

TABLE 3

AVERAGE COLONY DIAMETER (LINEAR GROWTH) OF ISOLATES OF *DIPLODIA* AND RELATED GENERA ON AGAR MEDIUM B SUPPLEMENTED WITH VARIOUS NITROGEN SOURCES

(Cultures incubated at 24 ± 2°C for three days)

Nitrogen source	Colony diameters (mm)						
	Species and isolate number						
	Botryodiplodia theobromae 44	*Diplodia macrospora* 35	*Diplodia natalensis* 147	*Diplodia natalensis* 157	*Diplodia natalensis* 213	*Diplodia zeae* 130	*Diplodia* sp. 19
Medium B (control)	69	10	84	86	73	22	75
Medium B plus:							
DL-alanine	79	10	90	69	82	21	79
L-arginine	90	13	90	90	90	18	90
L-asparagine	90	14	90	90	90	20	90
DL-aspartic acid	63	—	80	90	80	—	—
L-cysteine	80	6	90	74	77	10	69
L-cystine	75	10	90	84	76	22	81
DL-glutamine	88	11	90	90	90	23	90
DL-glutamic acid	71	11	90	86	90	2	90
Glycine	72	16	90	85	80	16	81
DL-histidine	83	15	90	90	87	14	90
L-hydroxy-proline	47	10	90	67	61	13	79
L-isoleucine	64	10	90	75	71	23	73
D-leucine	54	0	73	64	51	0	63
L-lysine	58	13	82	66	63	0	59
DL-methionine	72	10	90	87	73	32	74
D-phenyl-alanine	55	—	90	70	67	7	64
L-proline	90	—	90	90	90	25	90
DL-serine	81	0	90	90	83	21	85
D-threonine	62	20	84	69	56	19	61
D-tryptophane	33	6	57	31	41	0	50
DL-tyrosine	75	—	90	85	79	0	90
D-valine	67	11	83	79	72	18	71
L-valine	68	10	90	82	73	29	82
Casein hydro-lysate	90	13	90	90	90	41	90
Egg albumen	82	—	90	90	90	27	85
Gelatin	84	11	90	90	90	27	90
Protease pep-tone	90	21	90	90	90	42	90
Potassium nitrate	77	—	90	90	87	23	84
Potassium nitrite	46	0	57	39	47	12	39

Initial growth varied greatly among the isolates on BAM-A and on each of the different nitrogen compounds (table 2). Among the first 17 compounds, *Botryodiplodia theobromae* 44 grew more rapidly on only four than on BAM alone; *Botryosphaeria ribis* 55, however, did as well or better on 12 compounds; *Diplodia natalensis* 6 on nine, 107 on 10, 157 on nine, 213 on 10, and 230 on six. *Physalospora rhodina* 86 grew well on eight compounds. All isolates except 130 and 218 grew on BAM-A plus L-asparagine, L-proline, L-valine, casein hydrolysate, egg albumen, gelatin, protease peptone, and

TABLE 4

AVERAGE NUMBER OF PYCNIDIAL STROMATA AND PYCNIDIOSPORES PRODUCED BY ISOLATES OF *DIPLODIA* AND RELATED GENERA ON MEDIUM B SUPPLEMENTED WITH VARIOUS NITROGEN SOURCES

(Cultures incubated at 24°C for 30 days)

Nitrogen source	No. of pycnidia (per cm^3) and pycnidiospores (per plate $\times 10^3$)										
	Species and isolate no.										
	Diplodia natalensis						*Diplodia* sp. 19		*B. theobromae* 44		*Diplodia zeae* 130
	147		157		213						
	Pycnidia*	Pycnidio-spores†	Pycnidia	Pycnidio-spores	Pycnidia	Pycnidio-spores	Pycnidia	Pycnidio-spores	Pycnidia	Pycnidio-spores	Pycnidio-spores
Basal medium (control)	19	16	13	7	3	6	12	80	13	4	51
Basal medium plus:											
DL-alanine	19	40	11	<1	4	19	19	17	3	30	0
L-cysteine	25	0	21	0	24	0	29	0	20	2	0
L-cystine	33	22	71	0	10	25	30	37	12	8	29
Glycine	26	791	6	45	5	248	14	461	12	216	1,184
L-isoleucine	14	348	17	88	6	100	33	582	2	126	311
D-leucine	0	0	11	98	17	8	47	120	9	<1	0
DL-methionine	17	161	37	50	46	111	37	237	29	13	379
DL-serine	—	36	11	115	15	200	22	470	18	193	899
D-threonine	11	<1	13	<1	46	7	11	27	0	0	915
D-valine	0	0	12	7	0	0	22	27	0	0	0
L-valine	63	364	4	76	9	182	35	214	7	136	895
DL-aspartic acid	45	345	9	107	20	295	—	—	10	73	—
DL-glutamic acid	47	438	10	68	18	161	40	246	23	128	1,280
L-arginine	19	<1	20	15	11	246	93	484	15	117	463
DL-histidine	9	558	43	23	23	202	17	602	16	203	919
L-lysine	15	157	6	43	12	111	26	0	12	214	0
D-phenylalanine	23	371	6	62	17	105	42	600	7	120	287
DL-tyrosine	0	<1‡	14	77	16	130	60	373	4	41	1,216
L-hydroxyproline	11	452	13	112	4	225	17	307	11	180	781
L-proline	16	613	9	147	19	276	16	496	22	175	671
D-tryptophane	13	0	11	34	5	<1	10	44	28	180	0
L-asparagine	15	553	18	79	16	265	18	665	7	218	1,056
DL-glutamine	17	810	18	51	19	253	23	416	7	185	1,319
Casein hydrolysate	38	—	14	114	35	207	17	545	19	193	832
Egg albumen	47	483	9	34	9	264	20	572	24	198	1,456
Gelatin	29	585	26	24	18	167	63	150	10	248	1,340
Protease peptone	91	460	28	67	25	293	38	460	6	183	1,247
Potassium nitrate	18	625	37	40	30	233	18	348	15	100	1
Potassium nitrite	44	476	49	15	22	225	16	305	6	111	60

* Average of $4cm^2$ chosen at random on each of three culture plates.
† Average of three culture plates.
‡ There were no pycnidia on the random sample taken from culture plates, but a few formed on the plate at the margins and produced a few spores.

potassium nitrate as well as, or better than on BAM-A alone.

D. macrospora 35 was slower to start growth on BAM-B than were all other isolates, even *D. zeae* 130. Initial growth of an isolate was in general better on BAM-B (which contained biotin) than on BAM-A (without biotin). BAM-B was also more highly buffered than was BAM-A. Isolates *B. theobromae* 44 and *D. natalensis* 213 grew a little more rapidly on BAM-A (table 2) than on BAM-B (table 3), whereas isolate *D. natalensis* 157 grew more rapidly on BAM-B. Otherwise, these isolates, with few exceptions, grew more rapidly on nitrogen compounds added to BAM-B (table 3) than on those added to BAM-A (table 2).

All species tested on BAM-A and BAM-B over the 30-day period utilized all nitrogen sources, except that isolates 130 and 218 failed to grow on potassium nitrite on BAM-A.

Pycnidia and pycnidiospore formation

Species of *Diplodia* and *Diplodia*-like fungi varied in the formation of pycnidia and pycnidiospores on different sources of nitrogen (table 4; figs. 1 to 5). The actual number of pycnidia per unit of culture area was difficult to count on some media because of the aggregation of pycnidia and their formation in columnar stromata. The relative numbers of pycnidia formed singly in aggregation and, to some extent, in stromata columns are shown as follows: *D. natalensis* 147 (fig. 1), 157 (fig. 2), 213 (fig. 3); *Diplodia* sp. 19 (fig. 4); *B. thebromae* (fig. 5). The formation of columnar stromata is evident and may be taken into consideration in evaluating the placement or location of pycnidia with respect to substrate as shown in figures 2, 3, 5, and 7. Sporulation probably is more accurately estimated by number of spores (table 4). Several nitrogen sources were utilized by all species and produced good mycelial growth, but not all of the sources supported formation of pycnidia and pycnidiospores. *Diplodia* sp. 19, *B. theobromae* 44, *D. natalensis* 147, 157, 213, and *D. zeae* 130, grew on BAM-B alone and formed pycnidia and pycnidiospores. On BAM-B plus L-cysteine, all isolates except 44 formed pycnidia but did not form pycnidiospores, whereas only isolate 157 reacted in this way on L-cysteine. Isolate 157 produced pycnidia and only a few spores on DL-alanine, as did isolate 147 grown on D-threonine, L-arginine, and DL-tyrosine. Since all six isolates formed pycnidiospores on BAM-B alone but not on the medium with certain amino acids, these specific amino acids must have interfered with sporulation. Only isolates 19 and 157 produced pycnidia and pycnidiospores on D-valine, whereas all six isolates sporulated on L-valine. Apparently the D-light-rotation form of the amino acid inhibited sporulation.

Characters of isolates in culture

Species of *Diplodia* and *Diplodia*-like fungi have been differentiated by the morphological characters of mycelium, pycnidia, pycnidiospore, and stromata. Specific attention was therefore given to the effects of nitrogen nutrition on those characters, with particular attention to ones that remained unaltered. Characters traditionally described are those of isolates grown on BAM-B. Figures 1 to 5 show the gross culture appearances of isolates grown on BAM-B alone and on BAM-B plus each of the 29 nitrogen-containing amino acids and other compounds.

Mycelium. The only mycelial mats examined extensively were those of *B. theobromae* 44 and *D. natalensis* 213. When mycelium was transferred from basal medium into media containing amino acids and other nitrogen compounds, the new mycelial growth consisted of thin, delicate, and colorless

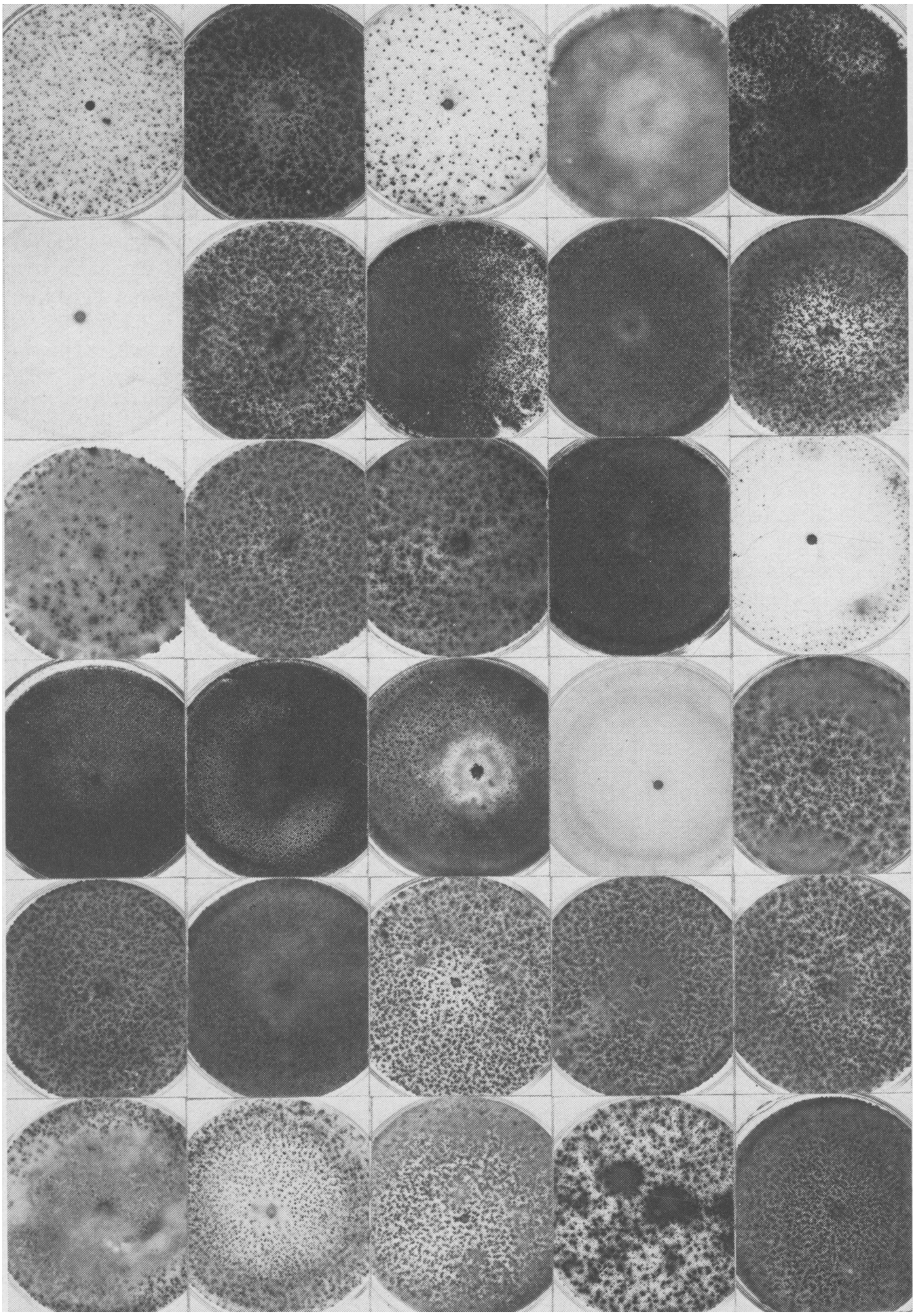

Fig. 1. Growth and sporulation of *Diplodia natalensis* 147 on basal medium (control) and on various nitrogen sources. Left to right, top to bottom: basal medium, glycine, DL-alanine, D-valine, L-valine, D-leucine, L-isoleucine, DL-serine, D-threonine, L-cysteine, L-cystine, DL-methionine, DL-glutamic acid, DL-aspartic acid, L-lysine, L-arginine, DL-histidine, D-phenylalanine, DL-tyrosine, D-tryptophane, L-proline, L-hydroxyproline, L-asparagine, DL-glutamine, casein hydrolysate, potassium nitrate, potassium nitrite, gelatin, egg albumen, and proteose peptone.

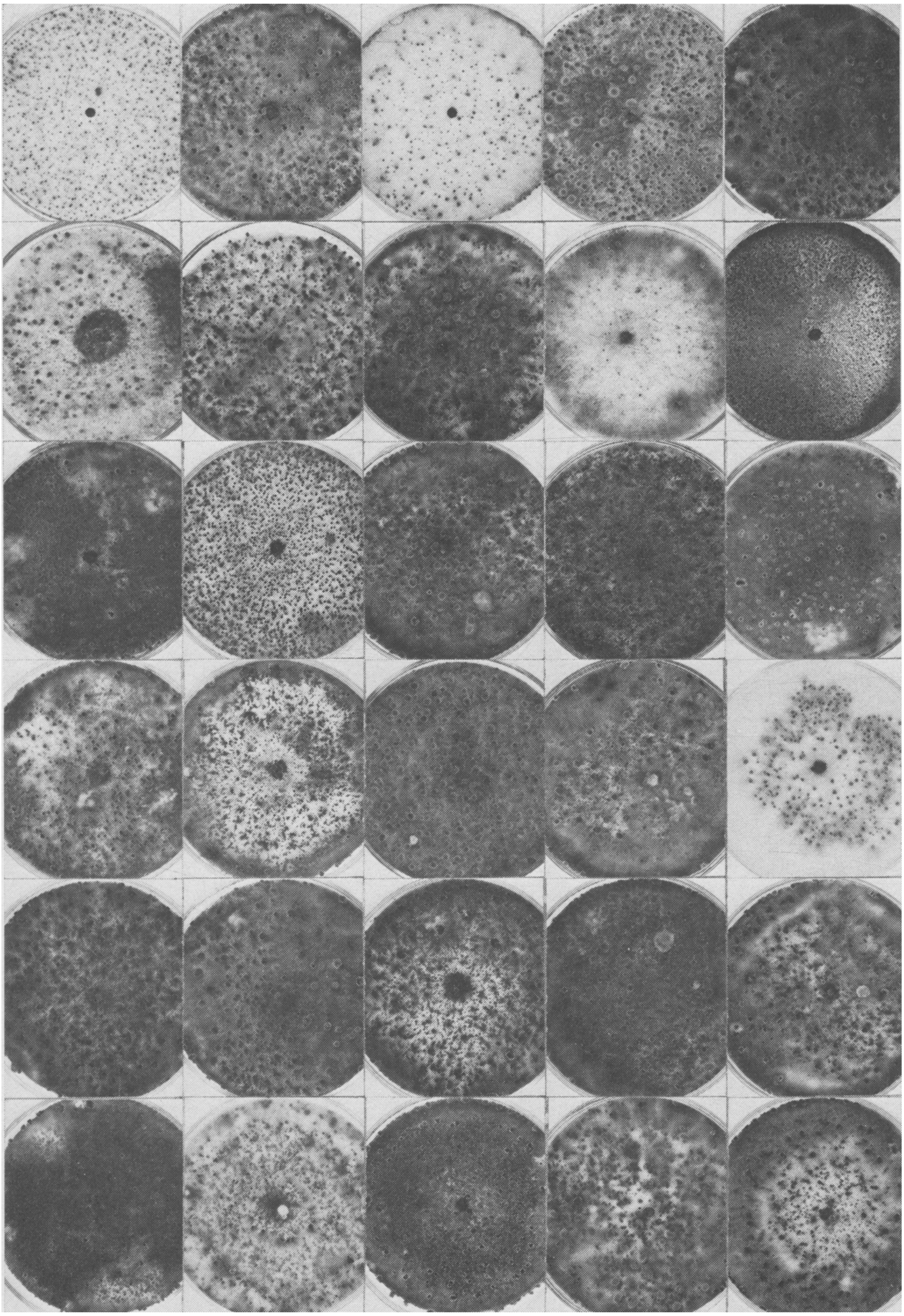

Fig. 2. Growth and sporulation of *Diplodia natalensis* 157 on basal medium (control) and on various nitrogen sources. Left to right, top to bottom: basal medium, glycine, DL-alanine, D-valine, L-valine, D-leucine, L-isoleucine, DL-serine, D-threonine, L-cysteine, L-cystine, DL-methionine, DL-glutamic acid, DL-aspartic acid, L-lysine, L-arginine, DL-histidine, D-phenylalanine, DL-tyrosine, D-tryptophane, L-proline, L-hydroxyproline, L-asparagine, DL-glutamine, casein hydrolysate, potassium nitrate, potassium nitrite, gelatin, egg albumen, and proteose peptone.

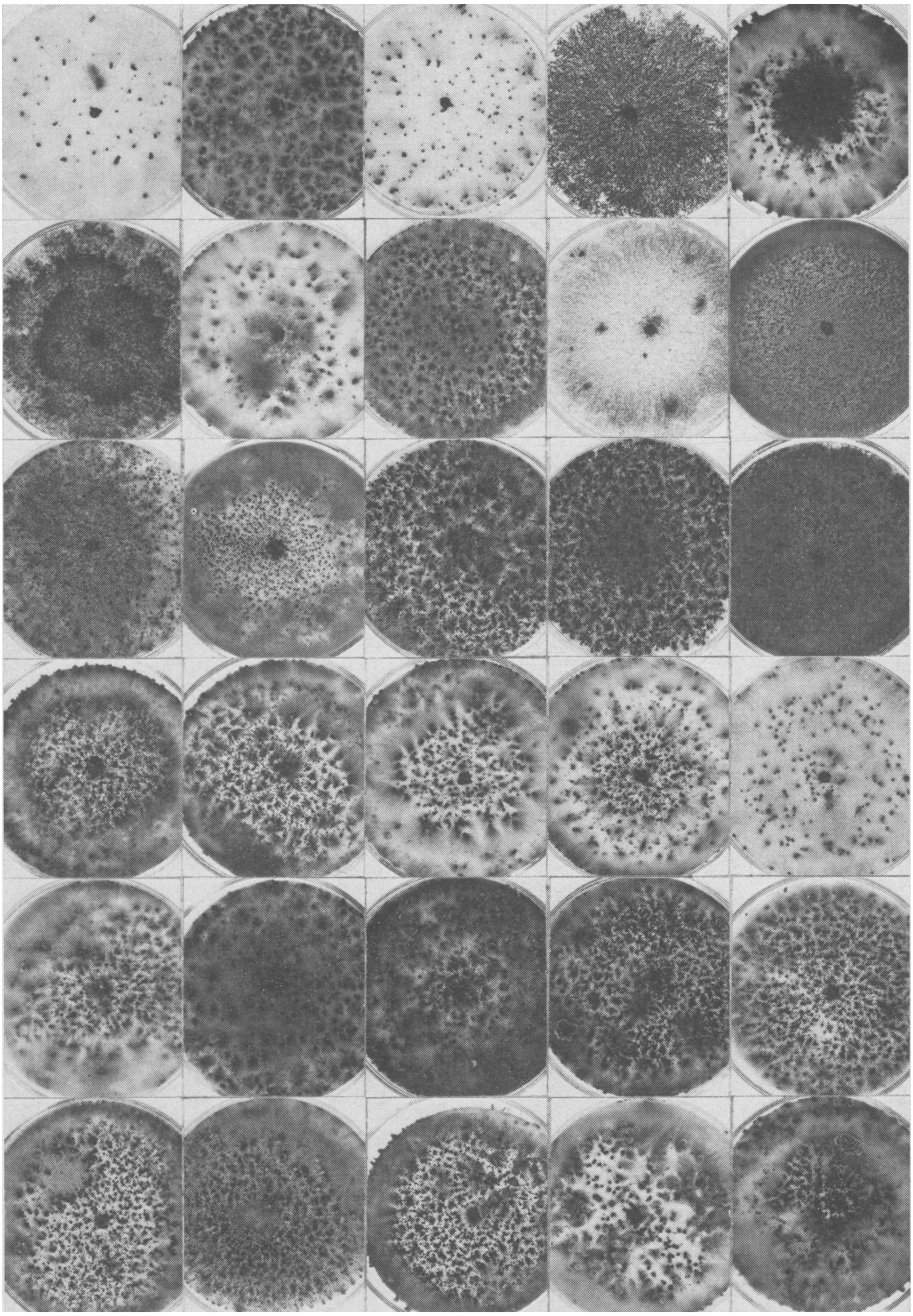

Fig. 3. Growth and sporulation of *Diplodia natalensis* 213 on basal medium (control) and on various nitrogen sources. Left to right, top to bottom: basal medium, glycine, DL-alanine, D-valine, L-valine, D-leucine, L-isoleucine, DL-serine, D-threonine, L-cysteine, L-cystine, DL-methionine, DL-glutamic acid, DL-aspartic acid, L-lysine, L-arginine, DL-histidine, D-phenylalanine, DL-tyrosine, D-tryptophane, L-proline, L-hydroxyproline, L-asparagine, DL-glutamine, casein hydrolysate, potassium nitrate, potassium nitrite, gelatin, egg albumen, and proteose peptone.

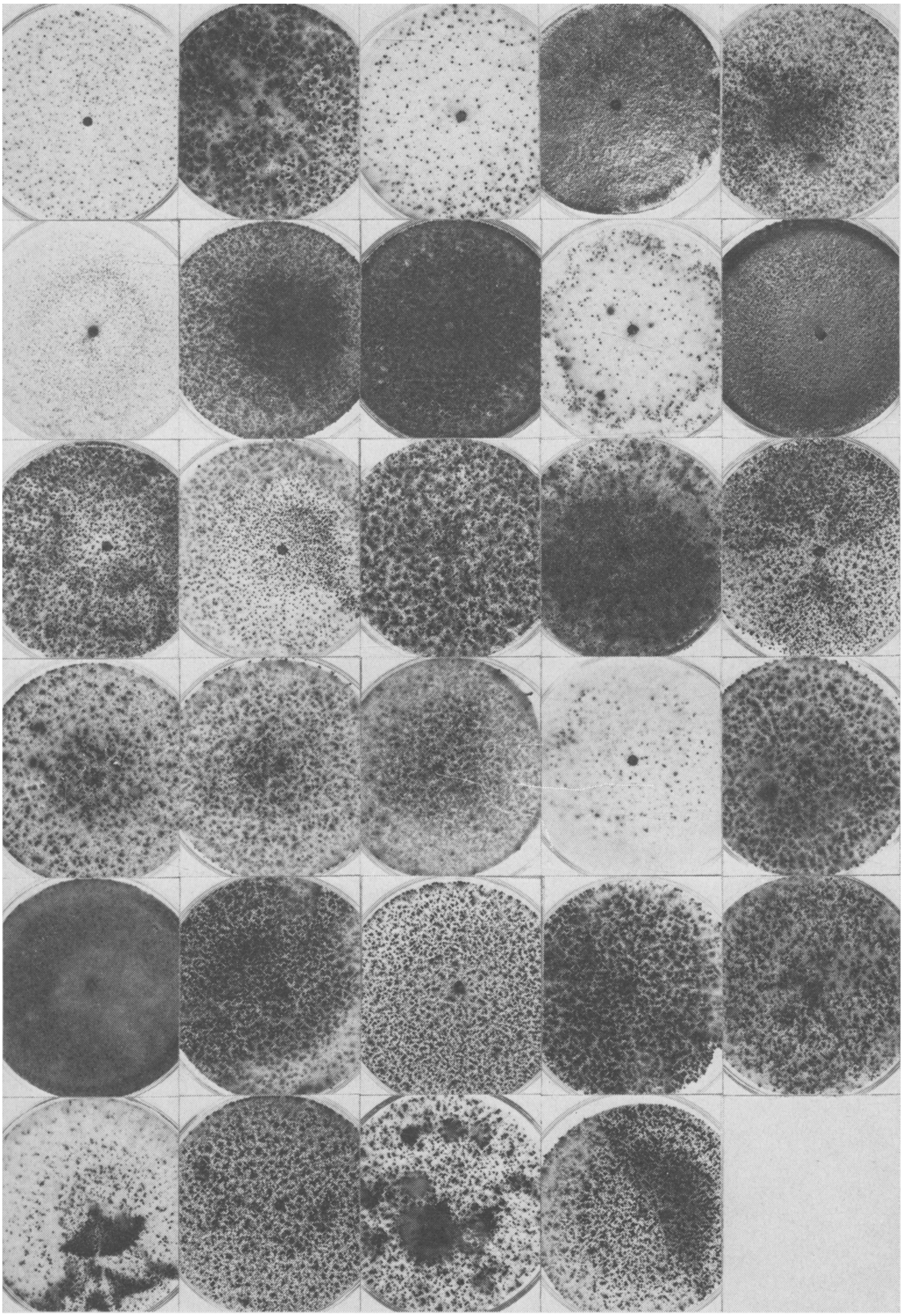

Fig. 4. Growth and sporulation of *Diplodia* sp. 19 on basal medium (control) and on various nitrogen sources. Left to right, top to bottom: basal medium, glycine, DL-alanine, D-valine, L-valine, D-leucine, L-isoleucine, DL-serine, D-threonine, L-cysteine, L-cystine, DL-methionine, DL-glutamic acid, DL-aspartic acid, L-lysine, L-arginine, DL-histidine, D-phenylalanine, DL-tyrosine, D-tryptophane, L-proline, L-hydroxyproline, L-asparagine, DL-glutamine, casein hydrolysate, potassium nitrate, potassium nitrite, gelatin, egg albumen, and proteose peptone.

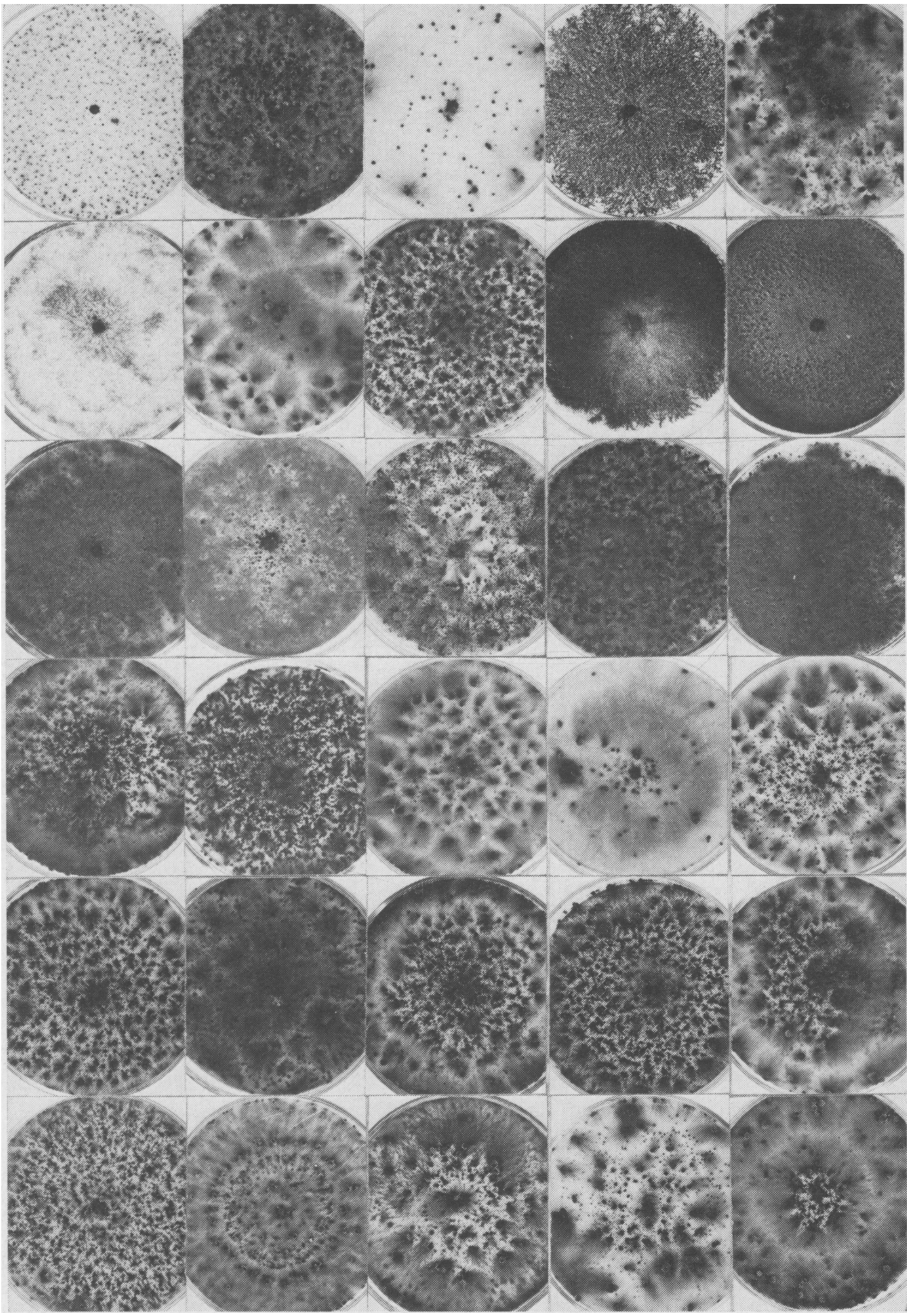

Fig. 5. Growth and sporulation of *Botryodiplodia theobromae* 44 on basal medium (control) and on various nitrogen sources. Left to right, top to bottom: basal medium, glycine, DL-alanine, D-valine, L-valine, D-leucine, L-isoleucine, DL-serine, D-threonine, L-cysteine, L-cystine, DL-methionine, DL-glutamic acid, DL-aspartic acid, L-lysine, L-arginine, DL-histidine, D-phenylalanine, DL-tyrosine, D-tryptophane, L-proline, L-hydroxyproline, L-asparagine, DL-glutamine, casein hydrolysate, potassium nitrate, potassium nitrite, gelatin, egg albumen, and proteose peptone.

hyphae. On most of the nitrogen compounds the morphology of the hyphae appeared to be normal. On D-valine (Brown, 1957), however, the hyphae converted into chlamydospores that were essentially swollen portions of the septate hyphae (fig. 6, A). Portions of hyphae enlarged, and all the protoplasm passed into them. When they reached a certain rather uniform size, the cell wall thickened and the hyphae became darkly colored. The empty hyphae appeared quite colorless. On DL-methionine and L-isoleucine the hyphae of the same isolate 44 mostly retained their shape, but numerous unilateral and terminal swellings were formed. The swellings had a thin wall and were colorless (fig. 6, B).

Although mycelium pigmentation was altered to some extent by the various nitrogen compounds combined with BAM-B, the range of pigmentation was not great: pale mouse-gray, mouse-gray, smoke-gray, blackish-mouse-gray, deep grayish-olive, iron-gray, and pallid quaker drab. (Color descriptions are from Ridgeway, 1912.) Colony color appeared to be affected to the greatest extent on the basal medium. Isolates of *D. natalensis* 157 and 213 and *D. theobromae* 44 were dark on BAM-A, and in shades of gray on BAM-B. The color formed on the basal medium appeared to dominate when supplemented with the different sources of nitrogen. Different isolates of the same species varied in color on the same nitrogen supplement. For example, *D. natalensis* 157 produced light mouse-gray, mouse-gray, dark mouse-gray, and deep mouse-gray on, respectively, D-tryptophane, DL-serine, D-valine, and L-asparagine. In contrast, isolate 213 of *D. natalensis* produced light olive-gray, olive-gray, dark olive-gray, deep olive-gray, mouse-gray, black mouse-gray, olivaceous black, and grayish olive pigment on, respectively, L-valine, L-arginine, L-lysine, D-phenylalanine, D-tryptophane, D-valine, D-leucine, and potassium nitrite.

Pycnidia. Pycnidial shape varied somewhat with nitrogen source. Simple pycnidia were typically globose with an ostiole, whereas pycnidia grouped in stromata were variable in shape. Pycnidia produced in or on the basal medium by isolates 19, 44, 147, 157, and 213 were mostly typically flask-shaped structures. Pycnidia produced by an isolate of *D. zeae* 130 were of two types: (1) submerged, globose, with a short ostiole; and (2) superficially globose to elliptical without noticeable ostioles. Pycnidia formed in a stromata were irregular in shape and often multiloculate (fig. 7).

Because single pycnidia were difficult to measure in most cultures, the smallest unit was measured—either single pycnidia or small clumps of pycnidia coalesced into stromata. The diameters of 30 fruiting-structure units (individual pycnidia insofar as could be determined) were measured on each of five cultures of each isolate on BAM-B and on the BAM-B supplemented separately with 29 nitrogen compounds. Table 5 shows the mean width of the measurements.

The size of the fruit structure varied with nitrogen compound and among isolates of the same species (table 5). For example, *Diplodia* sp. 19 produced fruiting structures with the following measurements: 1,160μ on glycine; 960μ on L-proline; 700μ on DL-histidine; 410μ on L-cystine; and 280μ on DL-alanine. Pycnidial units of isolates of *D. natalensis* 147, 157, and 213 measured, respectively: 1,060, 1,190, and 190μ on glycine; 340, 470, and 730μ on the basal medium; 430, 450, and 670μ on L-cysteine; and 650, 1,560, and 1,170μ on L-arginine.

The status of pycnidia, i.e., whether single or grouped or in a stromata, varied among the isolates on the differ-

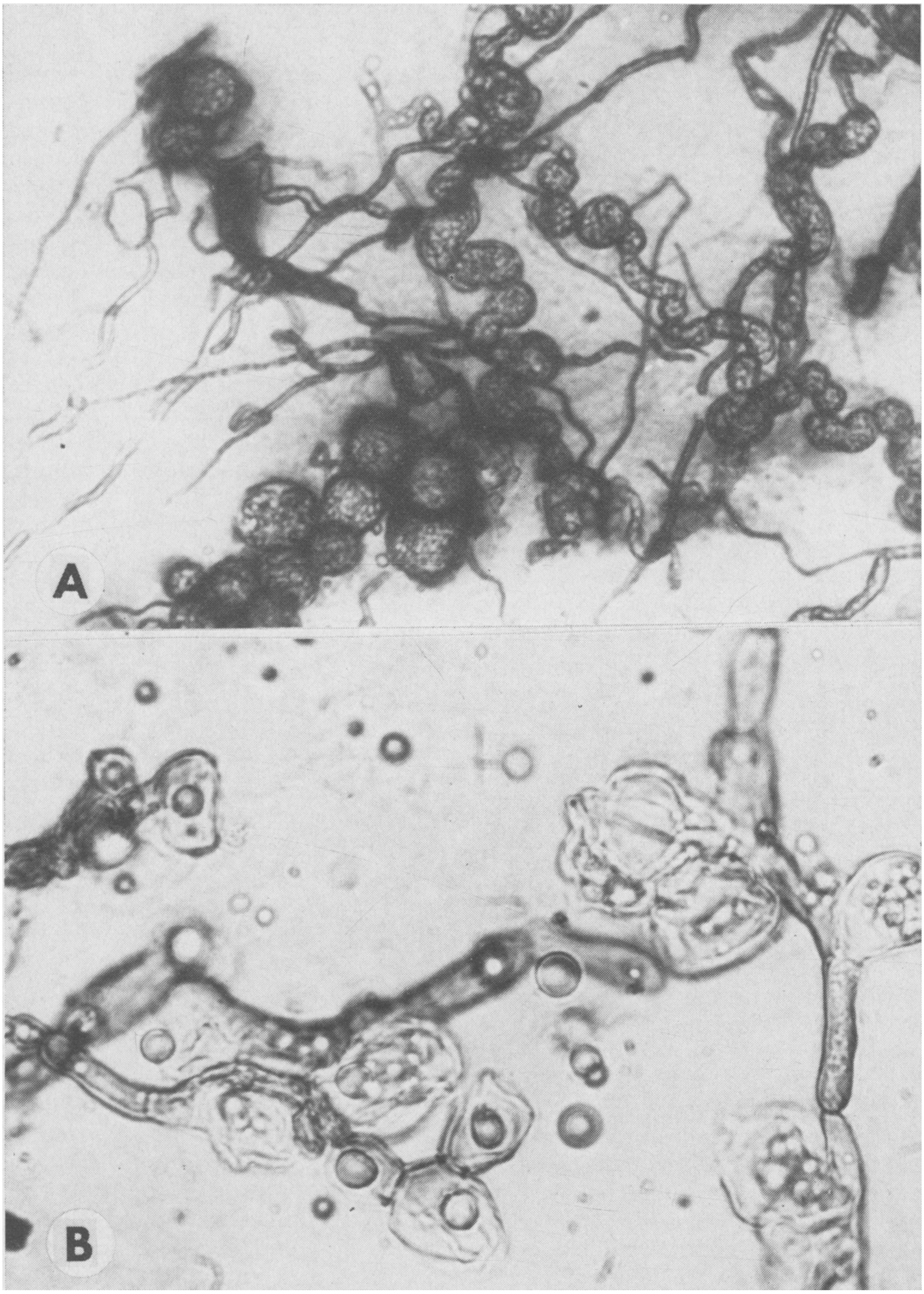

Fig. 6. Morphology of the mycelium: A, chlamydospores formed by *Botryodiplodia theobromae* 44 on D-valine; B, swellings formed on the hyphae of the same isolate grown on L-methionine.

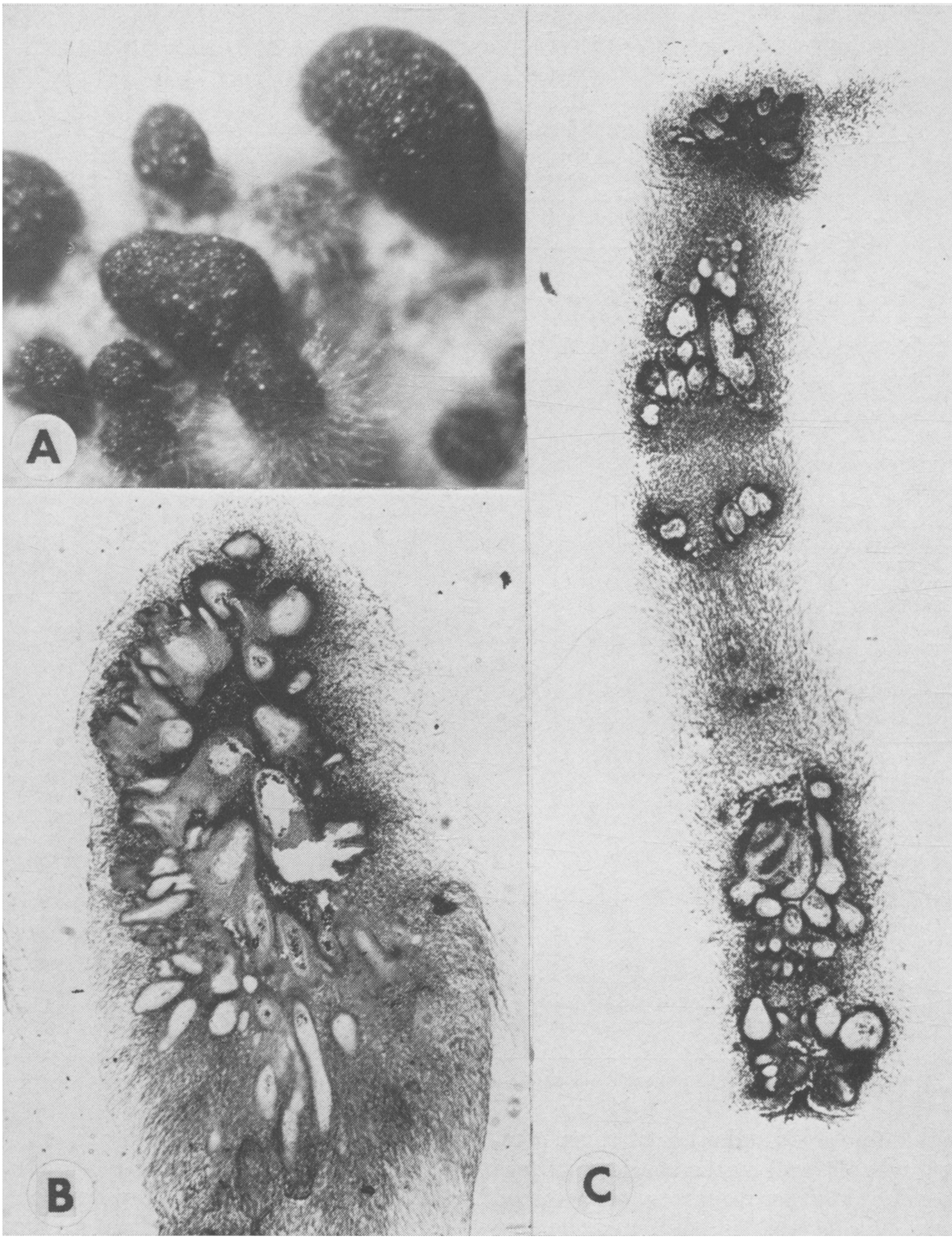

Fig. 7. Morphology of pycnidia and stromata of *Diplodia* and *Botryodiplodia*: A, superficial pycnidia of *D. zeae* 130, on the flat stromata; B, longitudinal section of stromata produced by isolate 157 of *D. natalensis* on medium B plus L-arginine; C, longitudinal section of stromata produced by isolate 44 of *B. theobromae* on medium B plus gelatin.

TABLE 5

AVERAGE* DIAMETERS OF PYCNIDIA OF *DIPLODIA* AND *DIPLODIA*-LIKE FUNGI GROWN ON MEDIUM B SUPPLEMENTED SINGLY WITH VARIOUS NITROGEN SOURCES
(Cultures incubated at 24°C for 30 days)

Nitrogen source	Diameters				
	Species and isolate number				
	Diplodia sp. 19	*Botryodiplodia theobromae* 44	*Diplodia natalensis*		
			147	157	213
	μ	μ	μ	μ	μ
Basal medium (control)	430	520	340	470	730
Basal medium plus:					
DL-alanine	280	1,050	460	600	710
L-cysteine	540	780	430	450	670
L-cystine	410	690	490	970	880
Glycine	1,160	1,590	1,060	1,190	190
L-isoleucine	600	230	740	1,210	1,180
D-leucine	410	350	—	1,120	730
DL-methionine	500	690	630	650	640
DL-serine	780	1,120	—	1,740	980
D-threonine	530	—	380	570	320
D-valine	440	430	—	1,000	—
L-valine	730	1,730	960	1,960	150
DL-aspartic acid	—	1,340	440	1,770	940
DL-glutamic acid	610	700	600	1,740	890
L-arginine	610	1,270	650	1,560	1,170
DL-histidine	700	1,530	650	930	810
L-lysine	740	1,040	1,100	1,970	800
D-phenylalanine	680	1,520	810	2,700	790
DL-tyrosine	700	1,380	—	860	970
L-hydroxyproline	580	1,310	650	1,460	1,500
L-proline	960	1,490	860	1,510	1,210
D-tryptophane	590	1,300	780	910	900
L-asparagine	720	1,470	670	1,260	710
DL-glutamine	790	1,470	810	1,440	1,000
Casein hydrolysate	810	1,440	1,050	1,140	850
Egg albumen	660	1,250	1,220	1,270	1,310
Gelatin	850	1,300	700	1,090	810
Protease peptone	750	2,460	720	930	940
Potassium nitrate	790	1,290	600	490	860
Potassium nitrite	680	1,190	620	430	720

* Average of 150 pycnidia or stromatal units with 30 from each of 5 separate cultures for each isolate on each medium.

ent amino acids and other nitrogen compounds. Several of the species of fungi produced either separate or grouped pycnidia as well as both separate and grouped, on the same medium and on different media supplemented singly with various nitrogen sources (tables 6 and 7). All isolates on BAM-A (table 6) and on BAM-B (table 7) produced pycnidia separately and uniformly distributed over the culture. The culture plates of isolates on BAM-B are shown in figures 1 to 5. Three isolates, *D. natalensis* 157 and 213 and *B. theobromae* 44, produced mostly separate pycnidia on BAM-A plus nitrogen compounds (table 6), but many more in groups and in stromata on BAM-B plus nitrogen sources (table 7). Pycnidia of *D. natalensis* 157 were grouped and single on two of 27 nitrogen compounds. This type of distribution was also true of isolate 157 on 18 of 29 compounds, and of isolate 213 on 10 of 29. *D. zeae*

TABLE 6

PYCNIDIAL CHARACTERS* OF ISOLATES OF *DIPLODIA NATALENSIS* AND *BOTRYODIPLODIA THEOBROMAE* ON MEDIUM A SUPPLEMENTED WITH VARIOUS AMINO ACIDS AND OTHER NITROGEN COMPOUNDS

(Cultures incubated for 30 days at ± 2°C)

Nitrogen source	*D. natalensis* 157				*D. natalensis* 213				*B. theobromae* 44			
	Stromata and pycnidia			Pycnidio-spore	Stromata and pycnidia			Pycnidio-spore	Stromata and pycnidia			Pycnidio-spore
	Loc.	Status	Hair		Loc.	Status	Hair		Loc.	Status	Hair	
Medium A (control)	SM	N	NH	—	SM	N	H	D	—	—	—	—
Medium A plus:												
DL-alanine	S	N	H	D	S	N	H	W	S	N	H	W
Glycine	S	N	H	W	S	N	H	D	S	GN	H	W
D-leucine	S	N	H	D	S	N	H	D	S	N	H	—
DL-methionine	M	N	—	—	SM	N	H	D	S	N	H	—
D-threonine	S	GN	H	W	S	N	H	D	S	N	H	—
L-valine	SM	GN	H	W	S	N	H	D	S	N	H	W
DL-aspartic acid	S	N	H	W	S	GN	H	D	S	N	H	—
DL-glutamic acid	S	N	H	D	S	GN	H	D	S	GN	H	—
L-arginine	S	N	H	W	S	N	H	W	—	—	—	—
DL-histidine	S	N	H	W	S	N	H	W	S	N	H	W
D-phenylalanine	S	N	H	D	S	N	H	D	S	N	H	—
DL-tyrosine	SM	GN	H	D	S	GN	H	D	S	N	H	—
L-proline	S	N	H	D	S	N	H	D	S	N	H	—
D-tryptophane	SM	G	H	W	S	N	H	D	S	N	H	—
L-asparagine	S	N	H	W	S	GN	H	D	S	GN	H	—
Casein hydrolysate	S	N	H	D	S	N	H	D	S	N	H	D
Egg albumen	S	N	H	D	S	N	H	D	S	N	H	—
Gelatin	S	N	H	D	S	N	H	D	S	N	H	W
Protease peptone	S	N	H	D	S	N	H	D	SM	N	H	D
Potassium nitrate	S	N	H	D	S	N	H	D	S	N	H	W
Potassium nitrite	SM	N	H	D	S	N	H	D	S	GN	H	W
DL-serine	—	—	—	—	S	N	H	D	S	N	H	—
L-lysine	—	—	—	—	S	N	H	—	S	N	H	—

* S, M, SM = superficial; submerged; superficial and submerged.
G, N, GN = grouped; not grouped; grouped and not grouped.
H, NH = hairy; not hairy.
D, W, DW = dry; in a wet matrix; dry and in a wet matrix.
— = not observed, or not grown on substrate.

TA

PYCNIDIAL CHARACTERS* OF ISOLATES OF *DIPLODIA* AND *BOTRYODIPLOI*
NITROG
(Cultures incubated

Nitrogen source	Specie							
	D. natalensis 147				*D. natalensis* 157			
	Stromata and pycnidia			Pyc-nidio-spore	Stromata and pycnidia			
	Loc.	Status	Hair		Loc.	Status	Hair	
Medium B (control)	M	N	NH	D	SM	N	H	
Medium B plus:								
DL-alanine	SM	N	NH	D	SM	N	H	
L-cysteine	SM	N	NH	—	SM	N	NH	
L-cystine	SM	N	NH	—	SM	N	H	
Glycine	—	—	—	—	S	N	H	
L-isoleucine	M	N	NH	D	S	GN	H	
D-leucine	—	—	—	—	S	GN	NH	
DL-methionine	M	N	NH	D	SM	GN	NH	
DL-serine	M	N	NH	D	S	GN	H	
D-threonine	SM	N	NH	—	SM	N	H	
D-valine	M	N	NH	—	S	N	H	
L-valine	M	N	NH	D	S	N	NH	
DL-aspartic acid	SM	N	NH	DW	SM	N	H	
DL-glutamic acid	SM	N	NH	D	SM	GN	H	
L-arginine	SM	N	NH	D	SM	GN	NH	
DL-histidine	SM	GN	NH	DW	SM	GN	H	
L-lysine	SM	N	NH	—	SM	GN	H	
D-phenylalanine	SM	N	NH	D	SM	GN	NH	
DL-tyrosine	SM	N	NH	W	SM	N	NH	
L-hydroxyproline	SM	N	NH	D	S	GN	H	
L-proline	SM	N	NH	D	SM	N	H	
D-tryptophane	—	—	—	—	S	GN	H	
L-asparagine	SM	N	NH	D	SM	GN	H	
DL-glutamine	SM	N	NH	D	S	GN	H	
Casein hydrolysate	SM	N	NH	D	SM	GN	H	
Egg albumen	SM	N	NH	D	SM	N	H	
Gelatin	SM	N	NH	D	S	GN	H	
Protease peptone	SM	N	NH	D	SM	GN	NH	
Potassium nitrate	M	GN	NH	D	S	GN	H	
Potassium nitrite	SM	N	NH	D	SM	GN	NH	

* S, M, SM = superficial; submerged; superficial and submerged.
G, N, GN = grouped; not grouped; grouped and not grouped.
H, NH = hairy; not hairy.
D, W, DW = dry; in a wet matrix; dry and in a wet matrix.
— = not observed, or not grown on substrate.

130 had a strong tendency to produce pycnidia grouped mostly in stromata. This isolate produced single pycnidia only on BAM-B and BAM-B with added glycine, DL-tyrosine, or L-hydroxyproline. In these respects, *Diplodia* sp. 19 reacted like *D. natalensis* 147, producing mostly single pycnidia, with grouped and single only on DL-histidine and potassium nitrate.

Orientation of the pycnidia with respect to the agar media also varied with nitrogen source (tables 6 and 7). Pycnidia were observed either submerged (fig. 7, B, C) or superficially on the stromata (fig. 7, A). Most pycnidia of *D. natalensis* and *B. theobromae* were embedded in the stromata, whereas those of *D. zeae* were both embedded and superficial. On BAM-A with nitrogen compound added (table 6), isolates 157, 213, and 44 produced pycnidia mostly on the agar surface, whereas on BAM-A without nitrogen compounds,

MEDIUM B SUPPLEMENTED WITH VARIOUS AMINO ACIDS AND OTHER
MPOUNDS
days at 24 ± 2°C)

te number

D. natalensis 213				*D. zeae* 130				*Botryodiplodia theobromae* 44				*Diplodia* sp. 19			
tromata and pycnidia			Pyc-nidio-spore	Stromata and pycnidia			Pyc-nidio-spore	Stromata and pycnidia			Pyc-nidio-spore	Stromata and pycnidia			Pyc-nidio-spore
oc.	Status	Hair		Loc.	Status	Hair		Loc.	Status	Hair		Loc.	Status	Hair	
SM	N	H	D	M	N	NH	D	SM	N	H	—	M	N	NH	D
SM	N	H	D	—	—	NH	—	SM	N	H	D	SM	N	NH	D
SM	—	H	—	—	—	—	—	SM	GN	H	D	M	N	NH	—
SM	—	NH	D	—	—	—	—	S	N	H	D	M	N	NH	D
S	N	H	DW	M	GN	NH	W	S	N	H	D	SM	N	H	D
SM	—	NH	D	M	G	NH	—	SM	N	H	—	SM	N	H	D
—	N	—	—	—	—	—	—	M	N	NH	—	M	N	NH	D
S	N	NH	D	M	G	NH	W	—	—	NH	—	M	N	H	D
S	N	H	D	M	G	NH	W	SM	N	H	—	SM	N	H	D
—	N	NH	—	M	G	NH	W	SM	N	NH	D	SM	N	H	D
—	N	—	D	—	—	—	—	—	—	—	—	—	—	—	—
SM	N	NH	D	—	—	—	—	SM	GN	H	W	SM	N	H	D
SM	N	H	D	—	—	—	—	SM	GN	H	W	—	—	—	—
SM	GN	NH	D	M	G	NH	DW	SM	N	H	W	SM	N	H	D
S	N	NH	D	M	G	NH	W	SM	N	H	W	SM	N	H	D
S	N	H	D	M	G	NH	W	S	N	NH	—	SM	GN	H	D
S	N	NH	D	—	—	—	—	SM	N	H	D	M	N	NH	D
SM	N	H	D	M	G	NH	D	SM	N	NH	W	SM	N	H	D
S	GN	H	D	M	GN	NH	DW	S	N	NH	—	SM	N	H	D
SM	N	H	D	M	GN	NH	W	S	N	H	—	M	N	NH	D
SM	GN	H	D	M	G	NH	DW	SM	N	H	W	M	N	NH	D
SM	N	H	DW	—	—	—	—	SM	GN	H	D	M	N	NH	D
S	GN	H	DW	M	G	NH	DW	S	N	H	W	SM	N	H	D
S	GN	H	DW	M	G	NH	DW	SM	GN	H	—	M	N	NH	D
S	GN	H	D	M	G	NH	DW	S	GN	H	—	SM	N	H	D
S	GN	H	W	M	G	NH	DW	SM	GN	NH	—	SM	N	H	D
M	GN	H	D	M	G	NH	W	SM	GN	NH	W	SM	N	NH	D
M	GN	H	W	M	G	NH	W	SM	GN	H	W	M	N	NH	D
S	GN	H	D	—	—	—	—	S	N	NH	W	SM	GN	NH	D
M	N	H	DW	—	—	—	—	S	N	NH	W	SM	N	NH	D

more of the pycnidia produced by the same isolates were submerged in the agar. Pycnidia of *D. zeae* 130 produced on BAM-B with added nitrogen were all submerged.

The presence or absence of hairs around the pycnidia or stromata was also influenced by the nitrogen supplements (tables 6 and 7). On BAM-A, pycnidia of *D. natalensis* 157 and 213, and *B. theobromae* 44 generally lacked hairs, whereas on BAM-B presence or absence of hairs varied with isolate and compound (table 6). Isolate 157 had naked pycnidia on BAM-A alone, but hairy pycnidia with each of the added nitrogen compounds (table 6). In contrast, on BAM-B alone (table 7) 157 had hairy pycnidia, but with the different added nitrogen compounds the pycnidia were hairy on 20 compounds but not on the other nine. Pycnidia of *D. zeae* 130 never had hairs on BAM-B either alone or with any of the added nitrogen compounds.

It is interesting that *D. natalensis* 147, 157, and 213 differed in hairiness on some of the same compounds.

TABLE 8

AVERAGE* DIMENSIONS (μ) OF PYCNIDIOSPORES OF SPECIES OF *DIPLODIA* AND RELATED GENERA GROWN ON MEDIUM B SUPPLEMENTED SINGLY WITH VARIOUS NITROGEN SOURCES

(Cultures incubated at 24°C for 30 days)

Nitrogen source	Species and isolate no.											
	Diplodia sp. 19		*Botryodiplodia theobromae* 44		*Diplodia zeae* 130		*Diplodia natalensis*					
							147		157		213	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Basal medium B (control)	27	15	27	15	28	6	24	15	28	15	26	15
Basal medium B plus:												
DL-alanine	24	15	26	16	—	—	23	14	28	14	27	14
L-cysteine	—	—	27	15	—	—	—	—	—	—	—	—
L-cystine	26	14	28	15	—	—	25	14	—	—	27	15
Glycine	26	14	27	14	22	6	24	13	29	14	26	15
L-isoleucine	25	13	26	15	23	6	23	12	30	14	27	14
D-leucine	25	14	28	16	—	—	—	—	30	15	25	14
DL-methionine	27	14	25	14	23	6	24	13	29	14	27	15
DL-serine	26	14	26	14	25	6	23	13	31	15	25	15
D-threonine	26	13	—	—	24	6	24	12	25	14	28	15
D-valine	25	14	—	—	—	—	—	—	30	15	—	—
L-valine	26	13	27	16	22	6	24	14	31	15	27	14
DL-aspartic acid	—	—	26	16	—	—	22	13	30	15	27	14
DL-glutamic acid	26	14	26	15	28	6	25	14	30	14	26	15
L-arginine	23	13	27	14	23	6	22	12	27	15	24	15
DL-histidine	24	13	27	15	21	6	22	12	29	14	27	14
L-lysine	—	—	28	14	—	—	22	12	30	14	26	15
D-phenylalanine	25	15	27	15	27	6	22	14	29	15	27	15
DL-tyrosine	26	14	28	15	27	7	22	14	30	15	27	14
L-hydroxyproline	24	14	27	14	20	6	22	12	30	15	27	14
L-proline	25	13	27	15	24	6	23	13	31	15	27	14
D-tryptophane	25	14	28	15	—	—	—	—	31	14	27	15
L-asparagine	24	14	27	14	24	6	22	13	29	15	24	14
DL-glutamine	25	14	27	15	25	6	22	13	30	15	27	14
Casein hydrolysate	25	14	26	14	23	7	22	13	29	15	26	14
Egg albumen	23	13	27	14	24	6	23	14	28	15	28	14
Gelatin	25	12	25	14	21	6	21	13	29	14	26	14
Protease peptone	24	13	26	14	24	6	22	12	28	15	25	14
Potassium nitrate	22	14	29	14	21	6	22	14	30	15	25	14
Potassium nitrite	23	13	27	14	23	7	23	13	29	15	24	14

* Average of 50 spores for each isolate on each treatment.

Pycnidiospores. *Diplodia natalensis, Botryodiplodia theobromae,* and *Physalospora rhodina* produced pycnidiospores without septa on media containing certain nitrogen sources. For example, *D. natalensis* 157, supplemented singly with different nitrogen compounds, produced a mixture of one-celled and two-celled pycnidiospores in media containing D-threonine and L-arginine, whereas isolate 213 produced nonseptate spores on D-threonine. *B. theobromae* 44 produced nonseptate spores on media supplemented with DL-histidine, D-phenylalanine, or potassium nitrite. *P. rhodina* 86 produced nonseptate pycnidiospores on all nitrogen sources tested.

Pigmentation of the pycnidiospores was influenced by the various nitrogen sources. Spores varied from hyaline to honey, light brown, and dark brown. For example, spores of *D. zeae* 130 were honey to light brown in all nitrogen sources that supported their formation. *D. natalensis* isolate 157 produced a mixture of hyaline and light-brown spores on L-threonine and L-arginine, but very dark-brown spores on medium supplemented with D-phenylalanine.

Only pigmented, septate spores of *B. theobromae* 44 and *D. natalensis* 147, 157, and 213 had very characteristic striation, which was parallel to the long axis of the spores. Spores of *D. zeae* were nonstriated on all nitrogen sources tested.

Average lengths and widths of 50 spores of five isolates are listed in table 8. Spore size varied little with nitrogen sources. For example, the extreme spore lengths were 17 and 22μ for *Diplodia* sp. 19. The mean length of pycnidiospores ranged from 21 to 25μ for *D. natalensis* 147, from 25 to 31μ for 157, and from 24 to 28μ for 213. These measurements were less variable than those for pycnidiospores of *D. zeae* 130, which ranged from $20 \times 6\mu$, on L-hydroxyproline, to $28 \times 6\mu$ on BAM-B alone.

Release of wet or dry pycnidiospores from pycnidia varied with the nitrogen source (tables 6 and 7). With isolate 157 on BAM-B, for example, pycnidiospores were exuded dry with L-cysteine, L-isoleucine, D-leucine, or D-threonine, but in a wet matrix with D-valine, L-arginine, or DL-histidine. Both types were observed in the same plate, however, with glycine, DL-methionine, DL-serine, or DL-phenylalanine. With *Diplodia* sp. 19, pycnidiospores were extruded dry on all BAM-B with the different nitrogen compounds.

Stromata. The stromata observed in these experiments were of the two types described in the first paper of this series —columnar and globose to flat. Isolates of *D. natalensis* and *B. theobromae* formed both types, whereas *D. zeae* produced only short, somewhat globose stromata (fig. 7).

Nitrogen source influenced the production of stromata. For example, *D. natalensis* 213 on BAM-B formed nonstromatic pycnidia on DL-alanine, L-cysteine, and L-cystine, but formed stromata containing fertile pycnidia on glycine, DL-serine, and DL-glutamic acid. *B. theobromae* 44 produced simple pycnidia on DL-alanine, L-cysteine, glycine, and L-arginine, but produced stromata containing pycnidia on L-cysteine, L-valine, DL-asparatic acid, and gelatin.

DISCUSSION

The data show that *Diplodia* and *Diplodia*-like fungi can utilize a wide range of nitrogen-containing compounds, but with differing results. Isolates of the same species differed in growth response on the same nitrogen compound. Our results differed from those of Brown (1957), who stated that

Botryosphaeria ribis produced maximum growth in a liquid medium supplemented with glycine or asparagine. In our experiments, however, maximum dry weights were obtained with DL-leucine and L-histidine, followed by glycine. DL-asparagine was one of the least effective nitrogen compounds in supporting the growth of mycelium.

The inability of certain isolates to utilize some nitrogen sources within the first few days was probably due to the slow action on nonfunction of trans-aminase, amino acid oxidase, or deaminase enzyme activity in the short incubation period.

The basal liquid medium A (BLM-A) contained only salts and no nitrogen. Isolates grown on BLM-A alone were slow to start but developed reasonably well. There is little question but that many of the *Diplodia* and *Diplodia*-like fungi grown on this medium obtained nitrogen from the atmosphere. This assumption cannot be definitely established, however, until carefully controlled experiments are made with tagged nitrogen. Cultures on liquid media were not held long enough to permit formation of fruiting structures. The addition of nitrogen compounds resulted in increased growth as reflected in total weight of mycelium. *Diplodia zeae* 130 was the only isolate of 10 grown on BLM-A, that failed to increase growth both on BLM-A and on each of the nine nitrogen compounds. That isolate, a comparatively slow-growing fungus, produced more mycelium on only five of the amino acids than it did on the BLM-A alone. Evidently *D. zeae* 130 does not utilize some amino acids.

Agar in basal agar medium A (BAM-A) may have served as a possible source of nitrogen, according to Leal, Gallegly, and Lilly (1967). Growth of the different isolates on BAM-A alone was not extensive. However, the fungi did produce pycnidia and pycnidiospores on the base medium alone. Basal agar medium B (BAM-B) contained 4 ppm biotin and was perhaps a little more highly buffered than was BAM-A. The biotin may have served as an added source of nitrogen as well as having other effects on growth. Comparisons of growth effects between BAM-A and BAM-B are limited to only four isolates, 44, 157, 213, and 130, that were grown on both basal media. Isolates 157 and 213 grew more rapidly on BAM-B than on BAM-A, isolate 44 grew about equally on both, whereas isolate 130 grew more slowly on BAM-B.

Growth, as measured in colony diameter at three days, was generally more on each of the compounds added to BAM-B than on those added to BAM-A. A comparison of colony diameters of isolates 44, 157, and 213 grown on BAM-A and BAM-B, both with the same 23 nitrogen compounds, shows that the mean colony diameters of each isolate on all compounds were, respectively, 7, 8, and 10 mm more on BAM-B than on BAM-A. Apparently, the presence of biotin in BAM-B was beneficial to growth of these fungi.

Five species of *Diplodia* and related genera (isolates 6, 35, 55, 107, and 230) did not produce pycnidia on any of the nitrogen compounds. Some of these same isolates (e.g., 230), however, produced fruit structures and spores on carbon compounds (see first paper). This may have been due to the fact that certain compounds required for sporulation were not produced by the isolates on the specific nitrogen source.

Mix (1933) reported that the source of nitrogen influenced the formation of pycnidia and spores of *Phyllosticta solitaria* Ell. & Ev. The specificity of the nitrogen source was found to be greater for the production of spores than for the formation of pycnidia. Also, different isolates of this fungus responded differently to the various nitrogen sources. Of the various compounds tried, the most favorable for sporulation seemed to be potassium nitrate, with albumen second; asparagine and peptone seemed somewhat less favorable. Our results do not differ in principle from those of Mix (1933). That is, different isolates of the same fungus respond differently to the same medium.

The morphological characters of the mycelium, pycnidia, pycnidiospores, and stromata were unstable as a result of influence by the different nitrogen compounds. Such characters, therefore, are of little value in identifying *Diplodia*-like fungi except, perhaps, when the isolates are grown on a standard medium.

LITERATURE CITED

BROWN, IRVIN, F., JR.
1957. Influence of carbon and nitrogen on the growth of *Botryosphaeria ribis*. Phytopathology **47**: 451. (Abstract)

DRAKE, C. R., and L. D. MOORE
1967. The effect of various nitrogen sources on growth of *Botryosphaeria ribis* in vitro. Phytopathology **57**: 645. (Abstract)

LEAL, J. ANTONIO, M. E. GALLEGLY, and VIRGIL GREENE LILLY
1967. The relation of the carbon-nitrogen ratio in the basal medium to sexual reproduction in species of *Phytophthora*. Mycologia **59**: 953–64.

MIX, A. J.
1933. Factors affecting the sporulation of *Phyllosticta solitaria* in artificial culture. Phytopathology **23**: 503–24.

RIDGEWAY, R.
1912. Color standards and color nomenclature. Published by the author. Washington, D.C.: United States National Museum.

isolate each of *D. zeae, D. macrospora, Botryodiplodia theobromae, Botryosphaeria ribis, Physalospora rhodina,* and a *Sphaeropsis* sp. Isolates were grown in synthetic liquid media and on synthetic agar media, supplemented singly with the different sources of nitrogen. Nitrogen compounds influenced mycelial growth and pigmentation, pycnidial size and orientation with respect to the substrate, presence of hairs on pycnidia, morphology of pycnidia and stromata and pycnidiospores, and exudation of the pycnidiospores. For example, D- and L-valine stimulated vegetative growth, but only L-valine stimulated pycnidial production of most isolates. Lysine and tryptophane retarded the mycelial pigmentation of several isolates, but increased pycnidial production in most species. L-cystine, L-cysteine, D-leucine, and tryptophane inhibited pycnidiospore formation in some isolates of *D. natalensis.*

These data indicate that *Diplodia* and other related genera of fungi may use a wide diversity of nitrogen sources, but that the source of nitrogen may alter the taxonomic characters currently used to delimit this group of fungi.

III. Variation in *Diplodia natalensis* from Grape in California

Single-spore colonies originating from individual pycnidia were compared with each other and with those from different pycnidia from the same grape cane, different canes from the same vineyard, and different vineyards, to evaluate the natural range in variation and stability of taxonomic characters currently used to delimit *Diplodia natalensis* P. Evans. Pycnidia produced in colonies originating from the same sources varied significantly in production of setae, shape, size, loculation, production of paraphyses, and in distribution, i.e., whether single, clumped, or in stromata. Distinct colony types, based mainly on number and distribution of pycnidia and extent of stromata formation, were recognized, and in some cases, as many as four types originated from an individual pycnidium. Colony type *per se* is not considered to be useful for taxonomic purposes. Computer analysis of 70,973 pycnidiospores produced in culture revealed that those from a single pycnidium vary as much in length and width as do those from different collections. Most mature spores produced in culture were dark in color, uniseptate, and characteristically furrowed lengthwise. Biseptate spores were observed occasionally. Spores from cultures had a mean length of $24.77 \pm 2.05\mu$ and width of $12.26 \pm 1.19\mu$, whereas mean length and width of pycnidiospores produced on the canes were $23.25 \pm 2.34\mu$ and $12.03 \pm 1.16\mu$, respectively. Correlation of spore length to width was poor, with $R = .329$.

These results suggest that several genera now recognized in this group are congeneric, and indicate a great need for determination of the inherent variation that these fungi are capable of exhibiting.

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