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LOMA LINDA UNIVERSITY

Graduate School

THE NITROGEN NUTRITION OF *EMMONSIA* (MONILIALES, ALEURISMACEAE)

by

Gary L. Bradley

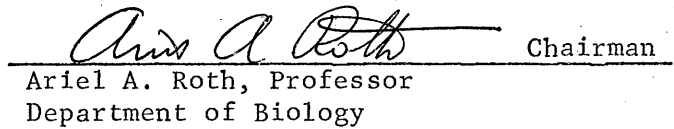
A Thesis in Partial Fulfillment

of the Requirements for the Degree

Master of Arts in the Field of Biology

June 1967

I certify that I have read this thesis and recommend that it be accepted as fulfilling this part of the requirement for the degree Master of Arts in the field of Biology.



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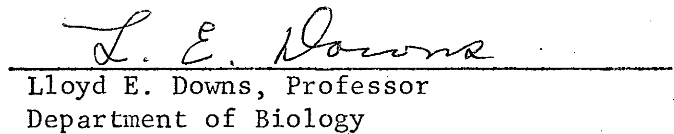

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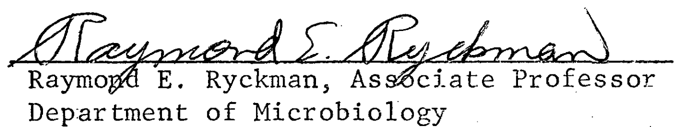

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INTRODUCTION

While studying Coccidioides immitis Rixford and Gilchrist (1896) in rodents, a new fungus was discovered, described, and placed in the genus Haplosporangium Thaxter (1914) with the specific name parvum Emmons and Ashburn (1942). In the lungs of the animals examined there was little or no host reaction to the spherical, nonbudding fungus cells. This organism has been found in a wide range of mammals, including man.

Carmichael (1951) investigated the strain and generic relationships of several isolates of H. parvum. He described four macroscopically different types of colonies; the granular type, the cottony type, the floccose type, and the glabrous type. The strains were divided into three groups based on colony type, relative growth rate, and spore size. The northern strains differed quite markedly from the southern strains in regard to spore size. In host tissues the northern strains reach a size of 300-400 microns while the southern strains reach a size of only about 10-40 microns.

Subsequently H. parvum was placed in a new genus, Emmonsia, with the specific name parva (Emmons and Ashburn, 1942) Ciferri and Montemartini (1959). Emmonsia belongs to the group (family) Moniliaceous Aleurismaceae (Aleurismae). Allied genera are Aleurisma, Glenosporella, Geomyces, and Staphylotrichum.

Emmonsia crescens (Emmons and Ashburn, 1942) Emmons and Jellison (1960) was segregated from E. parva. Colonies of E. crescens are indistinguishable from those of E. parva in culture at 30°C. However,

when incubated on nutrient medium at 37°C the conidia of *E. crescens* grow by spherical enlargement from their original size of 2.5-4.0 microns to a diameter of 250-400 microns. The wall may reach a thickness of 70 microns. *Emmonsia parva* will grow at 37°C in mycelial form, but when incubated at 40°C produces adiaspores comparable to its parasitic form. These reach a size of 10-25 microns with walls not over 2 microns thick. Adiaspores of *E. crescens* are multinucleate, while those of *E. parva* are uninucleate.

Other work has been done on the mode of infection and the pathology of this parasite. (Ashburn and Emmons, 1945; Dowding, 1947; Menges and Haberman, 1954; Battaglia, 1957)

Emmons (1964) induced budding in *E. crescens.* This organism germinates by formation of multiple germ tubes which probably result from the multinucleate nature of the spore. The adiaspores, which have reached a diameter not exceeding 200 microns will germinate if held 4-8 hours at room temperature. If such adiaspores are returned to 37°C (before germ tubes are visible), very young germ tubes are converted to spherical buds. Not all germ tubes bud, however. Budding is probably due to the migration of a nucleus into the young germ tube.

Ciferri and Montemartini (1959) did some nitrogen assimilation studies also. They used seven strains of *Haplosporangium parvum* and divided them into two groups: thermophilic and non-thermophilic. One hundred milliliters of Richard's solution was used in 200 ml Erlenmeyer flasks. To this was added the nitrogen source in the amount of 25 mg nitrogen. Growth was determined by averaging the dry weight of the mycelium in 3 replicate flasks after an incubation period of 25 days.

Fourteen nitrogen sources were tested and the results were somewhat varied. Their summation of results indicated peptone and urea to be the best nitrogen sources. Ammonium chloride was found to be approximately 1/3 as good and glycine, leucine, norleucine, alanine, phenylalanine, glutamic acid, and aspartic acid were 1/4 as good as peptone and urea. They also found arginine, histidine, proline, and nitrate to sustain little growth. A problem noted is that growth on urea varied quite markedly with the strain. Growth on arginine varied also with the strain. Since subsequent research separated these organisms into 2 species under a separate genus (*E. parva* and *E. crescens*) we might wonder if the differences were due to a difference in species or simply a strain difference. Also, since Richard's solution lacks many trace elements as well as thiamin and biotin the results could possibly be different on a more complete medium. There didn't seem to be any direct relationship between the thermophilic classification and the other ecological groups (northern and southern strains, etc.) established by Carmichael (1951).

Emmonsia is a parasite which is peculiarly well adapted for study of the host-parasite relationship. Several reasons can be listed for this. In the first place, this parasite has a life cycle which is uncomplicated by reproduction in the host tissues. Thus the physiology of the parasitic phase would be easier to study. Secondly, there appears to be no reaction to the parasite by the host tissues. Other items such as a wide variety of hosts, etc. could be listed. It was with the foregoing in mind that I decided to study further the nitrogen nutrition of the saprophytic phases of the two species of this genus

with the hope of answering some of the questions raised by the work of Ciferri and Montemartini (1959).

There has been much work done on the nitrogen nutrition of fungi. Table I shows the results of some of the recent work on a wide variety of fungi using a number of different nitrogen sources. Table II is an attempt to summarize growth results quantitatively on a yes-no basis. For those fungi with good growth reported using a particular nitrogen source I assigned 3 points, for fair growth I assigned 2 points, and for poor growth I assigned 1 point. In the qualitative studies yes was assigned 2 points and no assigned 0 point. Table II lists these sources in alphabetical order, but Table III lists the sources by descending point averages.

Notice that 16 nitrogen sources averaged out over 2.0 points. Of these 2 (glycylglycylglycine and norvaline) are based on only one fungus studied and 2 others (yeast extract and norleucine) are based on only 2 or 3 fungi studied. Among these good sources we find hydrolyzed casein, yeast extract and peptone. These obviously contain a complex mixture of amino acids. The single organic compounds which provide fairly good growth include arginine, asparagine, proline, alanine, glutamic acid, serine, and aspartic acid. The average of urea over the different fungi studied comes out fairly good although it varies from no growth to good growth. The ammonium ion is a fairly good nitrogen source also.

In Table IV I have taken the amino acids (since they are the most common nitrogen source) and the point averages tabulated in Table I and arranged them by the chemical groups in an attempt to discover

TABLE I (a)

Comparison of nitrogen sources for various fungi as found in recent studies

Key to species studied and references
1. Amoebidium parasiticum Whisler (1962)
2. Ascobolus immersus Yu-Sun (1964)
3. Blastocladia pringsceinii Craseman (1957)
4. Blastocladia ramosa Craseman (1957)
5. Blastomyces brasiliensis Gilardi (1965)
6. Blastomyces dermatitidis Gilardi (1965)
7. Botryosporium sp. Mehrotra (1964)
8. Candida albicans Gilardi (1965)
9. Cephaliophora tropica Weik and Pappelis (1964)
10. Cercospora viticola Sethi and Munjal (1963)
11. Cheatomium globosum McDonough and Rickard (1960)
12. Cladosporium cucumerium Strider and Winstead (1960)
13. Coccidioides immitis Gilardi (1965)
14. Colletotrichum coccodes Kurtz and Fergus (1964)
15. Colletotrichum inamdarii Hasija (1966)
16. Cryptococcus neoformans Gilardi (1965)
17. Darluca filum Nicholas and Villanueva (1965)
18. Diaporthe phaseolorum Bloss and Crittenden (1966)
19. Dipodascus aggregatus Batra (1963)
20. Dipodascus albidus Batra (1963)
21. Dipodascus uninucleatus Batra (1963) and Kuehn (1960)
22. Gonatobotrys simplex Whaley and Barnett (1963)
23. Helminthosporium carbonum Malca and Ullstrup (1962)
24. Helminthosporium turcicum Malca and Ullstrup (1962)
25. Hirsutella gigantea MacLeod (1959)
26. Histoplasma capsulatum Gilardi (1965) and McVeigh and Morton (1965)
27. Mortierella vinacea Chesters and Peberdy (1965)
28. Mucor rouxii Bartnicki-Garcia and Nickerson (1962)
29. Pestalotiopsis glandicola Bhargava and Tandon (1964)
30. Pestalotiopsis versicolor Agnihotri (1964)
31. Phlyctochytrium punctatum Goldstein (1960)
32. Phytophora cinnamoni Chee and Hewhook (1965)
33. Radiomyces embreei Embree (1963)
34. Radiomyces spectibilis Embree (1963)
35. Rhizophydium sphaerotheca Goldstein (1960)
36. Sporotrichum schenckii Gilardi (1965)
37. Trichurus spiralis Mehrotra (1964)
38. Zygorhynchus sp. Sarbhoy (1965)

TABLE I (b)

Key to results reported

<u>Quantitative studies</u>

G = good-67% or more of the maximum growth

F = fair-33-66% of maximum

P = poor-less than 33% of maximum

<u>Qualitative studies</u>

Y = yes growth occurs

N = no growth

TABLE I (c)

Source	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Species																		
Acetamide					Y	Y							Y						
a-alanine											G								
Alanine					Y	Y		Y		G			Y	G	F			G	G
p-aminobenzoic acid					N	N													
-aminobutyric acid																			
Arginine					Y	Y		Y						F				G	F
Asparagine	Y	G			Y	Y		Y	G	G	G	Y		F	G	Y	G		G
Aspartic acid		G			Y	Y		Y			G	Y		G	P				G
Cysteine					Y	Y						N							
Cystine								N					Y					F	N
Glutamic acid		G			Y	Y		Y			G		Y	F	F	Y			G
Glutamine		P			Y	Y										Y			
Glutathione					Y	Y													
Glycine					Y	Y	Y	Y	G		F		Y	G					P
Glycylglycylglycine														G					
Histidine					Y	Y		Y							P			F	G
Homocystine								N											
Hydroxylysine																			
Hydroxyproline					Y	Y		N											P
Isoleucine					Y	Y		Y						F				G	F
Leucine					Y	Y		Y			F			P	F				F
Lysine					Y	Y		Y			P								
Methionine					N	N		Y										F	G
Norleucine								Y											
Norvaline																			
Ornithine								N											
Phenylalanine					Y	Y		Y			P				G			G	
b-phenylalanine														F					
Proline					Y	Y		Y								Y		G	G
Serine					Y	Y		Y		G	F				G	Y			
Threonine					Y	Y		Y										G	
Tryptophane					Y	Y		Y	G					F					F
Tyrosine					Y	Y		Y					Y						
Urea		G						Y	G	G	G		Y	P					
Valine					Y	Y		Y		G	F			F	F			F	F
Ammonium salts	Y	P	Y	Y	Y	Y		Y	G		G		Y	P	F	Y	G		G
Nitrate salts	N			N			Y		G				Y	F	G	Y	Y		N
Nitrite salts													N	P	P		N		
Casein		P												P			G		G
Peptone									G					P			G		
Yeast extract																			

TABLE I (d)

Source	Species																	
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Acetamide																			
a-alanine						F						F				P			
Alanine	F	P				P			G	Y	G						G		
p-aminobenzoic acid							P										P		
-aminobutyric acid						P			F										
Arginine	G	P				F		G	F	Y	G	G				G	G		
Asparagine	F	P	N	G	G	P	G	G		Y	G			G	G				
Aspartic acid	G	P	N	G		F	G	G	P	Y	F						P		Y
Cysteine		N				P	P		P										
Cystine	N	N				P							N						
Glutamic acid	F	P	N	G		G	G		G	Y	G	G	Y			P	G	Y	Y
Glutamine				G								F	Y			G			
Glutathione																			
Glycine	N	P	N	F		P			F	Y	G						G		
Glycylglycylglycine																			
Histidine	P	P				P	P		P		G						P		
Homocystine																			
Hydroxylysine						P													
Hydroxyproline	N	P				P													
Isoleucine	N	N															G		
Leucine	F	P				P				Y	F						G		
Lysine		N				P	P	G	P								P		
Methionine	F	P				P	N		F		G	P				P			
Norleucine		Y															G		
Norvaline																	G		
Ornithine																	G		
Phenylalanine		Y					P		F			F	N			P	G		
b-phenylalanine																			
Proline	F	P				G	G		G										
Serine		Y				P			F		F								
Threonine						P			P	Y							G		
Tryptophane	N	F				P	P					P	N			P			
Tyrosine		N																	
Urea		N		G				G	F		F								
Valine	P	P				P					F						G		
Ammonium salts	F	F	N	G	P	F	G		F	Y	F	F		G	G	G	G		Y
Nitrate Salts	N	P	N	G	P	P	N	F	F	Y	F	F				P		Y	Y
Nitrite salts		N					N			N	P								
Casein	G	G	Y	G	G									G	G		G		
Peptone			Y							Y	G								
Yeast extract			Y			G													

TABLE II

Fungi nitrogen sources with point averages figured

Nitrogen Source	number of fungi studied	Total points	Average
Acetamide	3	6	2.00
a-alanine	4	8	2.00
Alanine	16	37	2.31
p-aminobenzoic acid	4	2	0.50
-aminobutyric acid	2	3	1.50*
Arginine	16	38	2.38
Asparagine	26	62	2.38
Aspartic acid	2	44	2.09
Cysteine	7	7	1.00
Cystine	8	5	0.62
Glutamic acid	25	56	2.24
Glutamine	8	17	2.12
Glutathione	2	4	2.00*
Glycine	18	33	1.84
Glycylglycylglycine	1	3	3.00**
Histidine	13	21	1.62
Homocystine	1	0	0.00**
Hydroxylysine	1	1	1.00**
Hydroxyproline	7	7	1.00
Isoleucine	9	6	0.67
Leucine	13	24	1.85
Lysine	10	14	1.40
Methionine	13	18	1.38
Norleucine	3	7	2.33*
Norvaline	1	3	3.00**
Ornithine	2	3	1.50*
Phenylalanine	13	24	1.85
b-phenylalanine	1	2	2.00**
Proline	11	26	2.36
Serine	11	23	2.09
Threonine	8	16	2.00
Tryptophane	13	19	1.46
Tyrosine	5	8	1.60
Urea	12	27	2.24
Valine	14	27	1.93
Ammonium	32	69	2.16
Nitrate	26	39	1.50
Nitrite	8	3	0.38
Casein (hydrolyzed)	12	31	2.58
Peptone	6	14	2.34
Yeast extract	2	5	2.50*

* based on 2 or 3 fungi ** based on 1 fungus

TABLE III

Point averages of nitrogen sources for fungi ranked from high to low

Nitrogen source	Point average
Glycylglycylglycine	3.00**
Norvaline	3.00**
Hydrolyzed Casein	2.58
Yeast extract	2.50*
Arginine	2.38
Asparagine	2.38
Proline	2.36
Peptone	2.34
Norleucine	2.33*
Alanine	2.31
Urea	2.24
Glutamic acid	2.24
Ammonium ion	2.16
Glutamine	2.12
Serine	2.09
Aspartic acid	2.09
Acetamide	2.00*
a-alanine	2.00
Glutathione	2.00*
b-Phenylalanine	2.00**
Threonine	2.00
Valine	1.93
Leucine	1.85
Phenylalanine	1.85
Glycine	1.84
Histidine	1.62
Tyrosine	1.60
-aminobutyric acid	1.50*
Ornithine	1.50*
Nitrate	1.50
Tryptophane	1.46
Lysine	1.40
Methionine	1.38
Cysteine	1.00
Hydroxylysine	1.00**
Hydroxyproline	1.00
Isoleucine	0.67
Cystine	0.62
p-aminobenzoic acid	0.50
Nitrite	0.38
Homocystine	0.00

* based on 2 or 3 fungi ** based on 1 fungus

TABLE IV

Amino acids listed by groups

Group	Amino Acid	Point Average
Aliphatic amino acids	Glycine	1.84
	Alanine	2.13
	Valine	1.93
	Leucine	1.85
	Isoleucine	0.67
Aromatic amino acids	Phenylalanine	1.85
	Tyrosine	1.60
	Tryptophan	1.46
Hydroxyamino acids	Serine	2.09
	Threonine	2.00
Imino acids	Proline	2.36
	Hydroxyproline	1.00
Basic amino acids	Lysine	1.40
	Arginine	2.38
	Histidine	1.62
Acidic amino acids	Aspartic acid	2.09
	Glutamic acid	2.24
Sulfur amino acids	Cystine	0.62
	Methionine	1.38
	Cysteine	1.00

any relationship between the extent to which a source can be utilized by fungi in general and the chemical structure.

A few observations can be made based on this literature. The sulfur amino acids seem to be rather poor nitrogen sources. The imino acids vary, as do the aliphatic amino acids. In general the basic and the aromatic amino acids are fair sources. The acidic amino acids and the hydroxyamino acids are fairly good nitrogen sources. The inorganic sources vary from ammonium: good, to nitrate: fair, to nitrite: poor.

MATERIALS AND METHODS

Emmonsia crescens was obtained from the American Type Culture Collection in lyophilized form. Malt extract broth was mixed with the fungus and spread on Sabouraud's medium in a Petri dish to germinate. Another E. crescens culture (same strain) was obtained from the ATC collection along with a culture of E. parva. Both of these cultures were grown on agar slants. Sub-cultures were transferred to Sabouraud's medium.

The colonies were checked both microscopically and macroscopically and found to be morphologically typical of Emmonsia.

Growth rates were obtained on solid media by innoculating a plate with a 2 mm disc of mycelium. Measurements were taken with a mm rule. Figure 1 shows the growth rate of E. crescens as determined by colony diameter.

Growth rates on liquid media were studied by floating 2 mm discs of mycelium on 25 ml of Sabouraud's liquid medium in a 125 ml Erlenmeyer flask. These were incubated in a Precision Scientific incubator at 25°C. Three cultures were collected every 2 days and the dry weight determined. Figure 2 shows the average dry weight of the mycelium against time. It seemed that the growth was fairly exponential up until about 14 days. It was thus determined to use an incubation period of 14 days.

The basal medium used in these experiments was a synthetic mixture of nutrients which was considered complete except for nitrogen. This basal medium was formulated after Kurtz and Fergus (1964) with

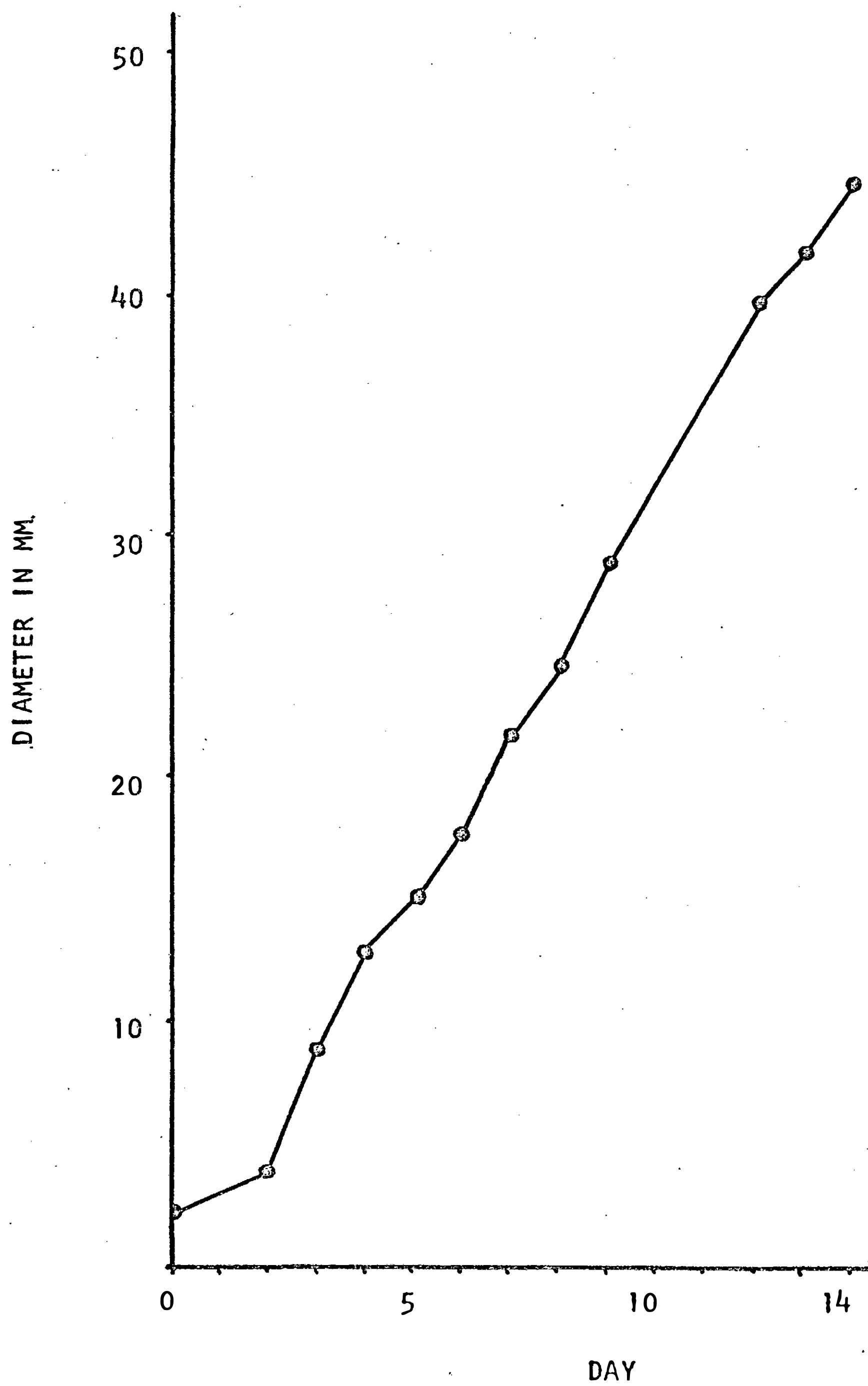

Fig. 1. Growth rate on Sabouraud's agar

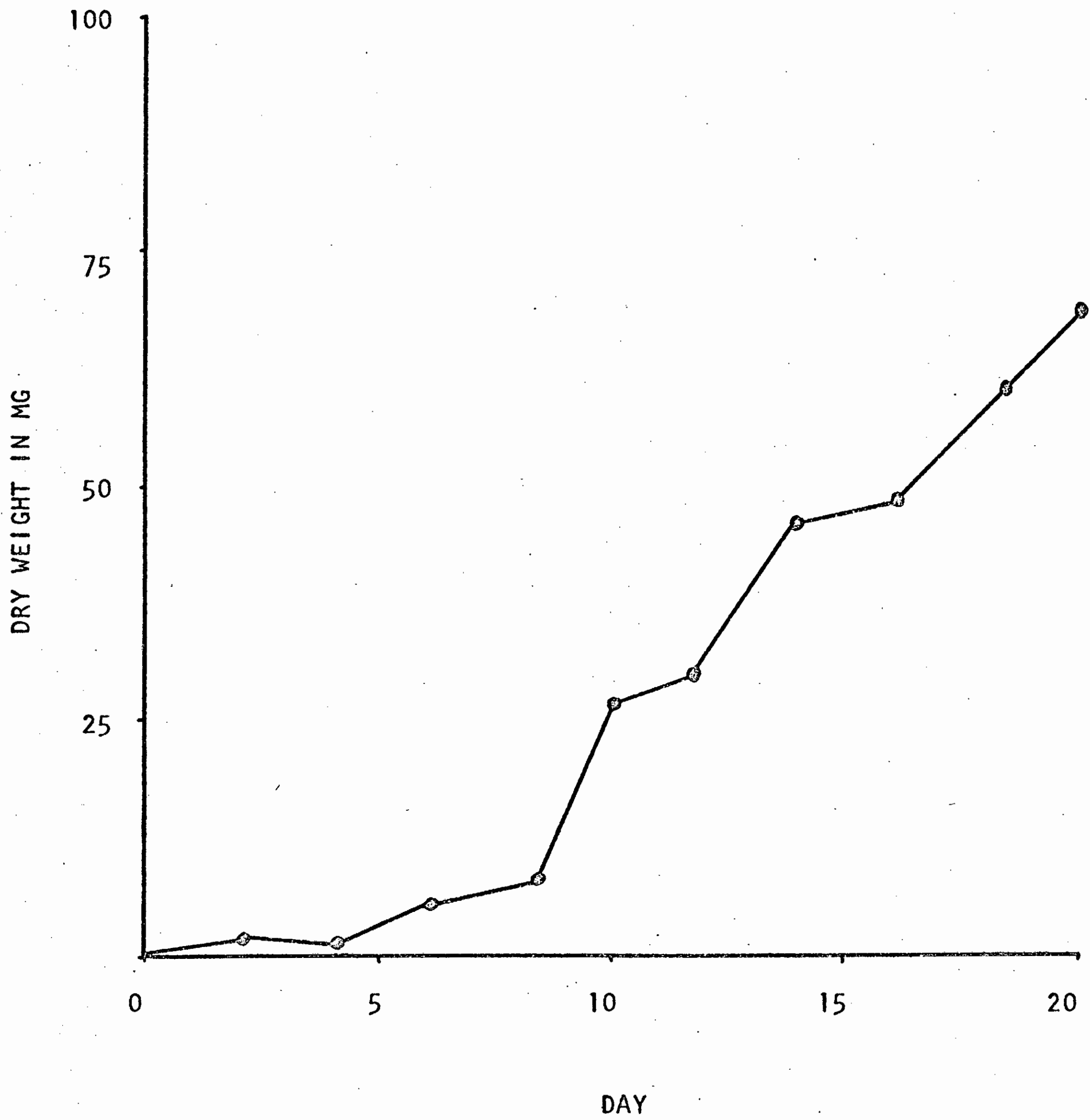

Fig. 2. Growth rate on Sabouraud's liquid

the exception of additional glucose and a more complete trace element mixture. Table V lists the composition of the basal medium.

TABLE V

Composition of the basal medium

Component	Amount
Magnesium sulfate (hydrated crystals).......	0.5 g
Potassium hydrogen phosphate...............	1.0 g
Glucose..................................	20.0 g
Thiamine.................................	0.1 mg
Biotin...................................	0.005 mg
Hydrogen borate..........................	0.025 mg
Zinc Sulfate (hydrated crystals).........	0.395 mg
Copper sulfate (hydrated crystals).......	0.090 mg
Manganese sulfate (hydrated crystals)....	0.030 mg
Sodium molybdate (hydrated crystals).....	0.025 mg
Iron (II) sulfate (hydrated crystals)....	0.140 mg
Distilled water..........................	1000 ml

To this basal medium any nitrogen source could be added.

Since Sabouraud's medium had been determined to support good growth by Emmonsia, it was used as a quantitative nitrogen standard. Sabouraud's medium contains 10 g peptone/liter of final solution. Peptone was assumed to be approximately 16% nitrogen. Thus Sabouraud's contains approximately 1.6 g of nitrogen/liter. This amounts to 0.04 g of nitrogen/25 ml. All sources of nitrogen were used in a concentration which would yield this amount of available nitrogen per flask. Nitrogen of L-amino acids was assumed to be totally available and the D-amino acid nitrogen was assumed to be unavailable.

In making up a nitrogen assimilation experiment, 5 replicates of each nitrogen source were prepared. The basal medium was mixed with the nitrogen source in a beaker and tested on a pH meter. The pH was then adjusted to 5.8 ± 0.2 with KOH or HCl. The medium was then measured into 25 ml portions and placed into the 125 ml Erlenmeyer flasks. These flasks were autoclaved at 15 pounds per square inch for 15 minutes. Discs of mycelium 2 mm in diameter were punched equidistant from the center of a colony. These discs (one per flask) were aseptically transferred and floated on the surface of the media in these sterile flasks.

After a 14 day incubation period the mycelial growth was collected and washed with distilled water on Whatman #2 filter paper. Each individual colony was placed in a weighing bottle and dried at $80^{o}C$ for 24-36 hours. The bottles were cooled in a dessicator and weighed on a Mettler analytical balance.

All the experiments in this paper were not done simultaneously. However, all *E. parva* experiments except those involving valine, cytosine and urea were done at the same time under the same conditions.

RESULTS

One of the first experiments was conducted to find a "complete" medium to serve as a maximal control. Cultures were grown using Sabouraud's liquid medium, basal medium plus peptone, and basal medium plus casein hydrolyzate. The results showed that casein provided the best growth and henceforth served as the "complete" control for subsequent experiments. Another experiment was conducted to test soil extract as a growth medium. It supported little growth. The minimal nitrogen source control medium consisted of basal medium alone.

When the mycelial discs were cultured on some media there was no evidence of growth. On other media, after the 14 day incubation period, slight evidence of growth was observed but was not sufficient to be significant. Only the discs which showed a substantially noticeable amount of growth were collected.

A question which might be raised is whether any other nitrogen sources were carried over with the innoculum and if so, whether this affected the rate of growth. In order to answer this question I set up an experiment in which I transferred to flasks of basal medium discs with various amounts of solid medium attached. The amounts ranged from little or no agar to a column 3 mm long and of the same diameter as the discs. In all flasks the growth was very slight and no significant difference was noted.

The results of the main body of experiments are shown in Table VI for _E. crescens_ and Table VII for _E. parva_. Rather than list the

data for all 5 replicates for each source, I have listed the mean, variance, and standard deviation.

TABLE VI

Results of nitrogen metabolism experiments on <u>Emmonsia crescens</u>

Source	Mean Dry Weight in Mg.	Variance*	Standard Deviation
Casein	242.77	48.31	6.95
Serine	94.66	148.62	12.19
Proline	79.40	176.86	13.29
Glycine	54.20	2855.24	53.43
Alanine	44.68	161.73	12.71
Isoleucine	43.04	19.24	4.38
Glutamic acid	42.30	107.77	10.38
Aspartic acid	31.30	21.34	4.62
Ammonium	26.40	110.64	10.51
Arginine	24.02	142.94	11.95
Urea	11.54	8.44	2.90
Leucine	9.77	27.80	5.27
Histidine	3.23	3.12	1.76
Adenine	slight growth		
Betaine	slight growth		
Choline	slight growth		
Cytosine	slight growth		
Lysine	slight growth		
Methionine	slight growth		
Nitrate	slight growth		
Threonine	slight growth		
Trimethylamine	slight growth		
Valine	slight growth		
Basal	no growth		
Caffeine	no growth		
Cysteine	no growth		
Phenyalanine	no growth		
Thymine	no growth		
Tryptophane	no growth		

* Variance = Standard deviation squared

TABLE VII

Results of nitrogen metabolism experiments on <u>Emmonsia parva</u>

Source	Mean Dry Weight in Mg.	Variance*	Standard Deviation
Casein	334.82	57.61	7.59
Proline	122.02	11,000+	105.42
Alanine	69.60	279.20	16.70
Arginine	52.96	119.79	10.94
Ammonium	39.74	110.19	10.49
Serine	22.04	20.83	4.56
Aspartic acid	21.80	0.72	0.84
Isoleucine	19.30	4.17	2.04
Glycine	15.92	23.94	4.89
Histidine	5.14	1.76	1.32
Adenine	slight growth		
Betaine	slight growth		
Choline	slight growth		
Cytosine	slight growth		
Glutamic acid	slight growth		
Leucine	slight growth		
Lysine	slight growth		
Nitrate	slight growth		
Phenylalanine	slight growth		
Threonine	slight growth		
Thymine	slight growth		
Trimethylamine	slight growth		
Urea	slight growth		
Valine	slight growth		
Basal	no growth		
Caffeine	no growth		
Cysteine	no growth		
Methionine	no growth		
Tryptophane	no growth		

* Variance = Standard deviation squared

DISCUSSION

In examining the data we need to keep in mind the questions which I have posed in this research. First, is there a significant difference in nitrogen source utilization by the two fungi, and second, what are the differences in nitrogen utilization within each of these species?

Of 29 nitrogen sources tested including the "complete" and basal media, *E. crescens* utilized 13 sources to a sufficient degree to measure. *E. parva* utilized 10 sources. Both species utilized several other nitrogen sources to a slight extent.

The basal medium alone, and with caffeine, cysteine, and tryptophane added supported no growth for either organism. Phenylalanine and thymine provided no growth for *E. crescens* while methionine supported none for *E. parva*. All other sources tested provided at least slight growth.

In comparing data between sources which supported enough growth to measure it would be helpful to analyze for significance of difference by using analysis of variance or a *t* test. The problem with either of these statistical methods is that the variances must be assumed to be homogenous. Upon examining Tables VI and VII it can be noted that the variances differ widely. The reason for these differences was not ascertained in this study. These differences might be due to inequalities in sample inoculum such as the number of potential growth sights available or genetic variation. Unknown exogenous factors

could also be significant. In consulting with a biostatistician, it was decided to divide the data into groups in which the variances would be within the proper range. According to him, the low variance to high variance ratio should be no more than approximately 1:8.

Table VIII shows the data of Tables VI and VII divided into 3 groups within each species. The only source which possibly lies outside the allowable variance range is E. parva aspartic acid, but statistician felt that it was close enough to be included within the group. Group 1 might be called the high variance group and Group 2 the low variance group. The third group includes those with such extremely high variances as to be questionable.

In comparing one source between the 2 species, a t test could be done only if they both lie within the same group (1 or 2). Serine is the only source which "crosses" groups. Since the variances of E. crescens serine and E. parva serine are closer than 1:8, a t test between them would be valid also. Table IX shows the results of t tests between E. crescens and E. parva. My data indicates a significant difference in utilization of arginine, alanine, casein, isoleucine, and serine between these 2 species of the genus Emmonsia. The difference in utilization of ammonium, aspartic acid, and histidine (according to my data) cannot be considered significant. Comparisons have not been made between the organisms when a source is utilized in one and not in another. The results in this case are obvious.

Analysis of the nitrogen source utilization within each species was also conducted. Table X shows the results of t tests between all sources for each species within each group. The results show significant

TABLE VIII

Organisms and sources grouped according to variances*

Group	Source	Mean	Variance
1. *E. crescens*	NH4	26.40	110.64
	ARG	24.02	142.94
	GLU	42.30	107.77
	PRO	79.40	176.86
	ALA	44.68	161.73
	CAS	242.77	48.31
	SER	94.66	148.62
2. *E. crescens*	LEU	9.77	27.80
	ASP	31.30	21.34
	ISO	43.04	19.24
	URE	11.54	8.44
	HIS	3.23	3.12
3. *E. crescens*	GLY	54.20	2855.24
1. *E. parva*	CAS	334.82	57.61
	ALA	69.60	279.20
	NH4	39.74	110.19
	ARG	52.96	119.79
2. *E. parva*	GLY	15.92	23.94
	ASP	21.80	.72
	ISO	19.30	4.17
	HIS	5.14	1.76
	SER	22.04	20.83
3. *E. parva*	PRO	122.02	11,000+

* Abbreviations used

ALA-alanine
ARG-arginine
ASP-aspartic acid
CAS-casein
GLY-glycine
GLU-glutamic acid
HIS-histidine
ISO-isoleucine
LEU-leucine
NH4-ammonium
PRO-proline
SER-serine
URE-urea

TABLE IX

Results of *t* tests comparing nitrogen sources for *E. crescens* and *E. parva**

	Source	*t*	df	% significance
Within Groups	NH4	1.56	4	80
	ARG	3.45	3	95
	ALA	3.36	4	95
	CAS	29.61	3	99.9
	ASP	11.70	1	90
	ISO	15.39	4	99.9
	HIS	1.44	2	70
Between Groups	SER	11.90	4	99.9

*Refer to Table 8 for abbreviations

differences between the sources within a group. Table XI summarized the results of Table X. Those sources which show no significant difference at the 95% level are connected by the underline. The sources at the extremes of a line are not significantly different by the *t* test, according to my data.

Due to differences in variances, the group 1 sources cannot be compared with the group 2 sources by the usual *t* test. These sources were compared using a *t* test formula in which variance differences are allowed for. When I made these tests between groups, no significant differences were demonstrated. The degrees of freedom on nearly every test was under 1.

In comparing the nitrogen utilization of *Emmonsia* with other fungi the data correlates quite well. We can, however, notice several interesting differences. The first nine nitrogen sources in Table VII

TABLE X

Results of *t* tests comparing nitrogen sources within species*

Species	Between	*t*	df	% significance
E. crescens	CAS-SER	15.06	3	99.9
	CAS-PRO	41.55	3	99.9
	CAS-ALA	35.92	3	99.9
	CAS-GLU	32.42	3	99.9
	CAS-NH4	37.99	3	99.9
	CAS-ARG	35.34	3	99.9
	SER-PRO	1.96	4	80.0
	SER-ALA	5.84	4	99.0
	SER-GLU	6.19	4	99.0
	SER-NH4	10.42	4	99.9
	SER-ARG	7.93	3	99.0
	PRO-ALA	5.29	4	99.0
	PRO-GLU	5.97	4	99.0
	PRO-NH4	17.98	4	99.9
	PRO-ARG	20.06	3	99.9
	ALA-GLU	.26	4	50.0
	ALA-NH4	2.96	4	95.0
	ALA-ARG	2.95	3	90.0
	GLU-NH4	3.08	4	95.0
	GLU-ARG	2.87	3	90.0
	NH4-ARG	5.61	3	98.0
	ISO-URE	13.83	4	99.9
	ISO-LEU	9.25	3	99.0
	ISO-HIS	33.20	2	99.9
	URE-LEU	1.18	3	60.0
	URE-HIS	10.00	2	99.0
	LEU-HIS	2.64	2	80.0
E. parva	CAS-ALA	25.05	4	99.9
	CAS-ARG	45.30	4	99.9
	CAS-NH4	53.23	4	99.9
	ALA-ARG	2.36	4	90.0
	ALA-NH4	2.90	4	95.0
	ARG-NH4	1.91	4	80.0
	SER-ISO	1.65	4	80.0
	SER-GLY	1.85	3	80.0
	SER-HIS	6.50	4	99.0
	ISO-GLY	1.13	3	60.0
	ISO-HIS	10.74	4	99.9
	GLY-HIS	4.11	3	95.0

* Refer to Table VIII for abbreviations

TABLE XI

Comparison of significance of nitrogen sources within species and groups. (For details consult text)

E. crescens Group 1

CAS	SER	PRO	ALA	GLU	NH4	ARG
	—	—	—	—	—	—
				—	—	—
			—	—		

E. crescens Group 2

ISO	ASP	URE	LEU	HIS
		—	—	
			—	—

E. parva Group 1

CAS	ALA	ARG	NH4
	—	—	
		—	—

E. parva Group 2

SER	ISO	GLY	HIS
—	—	—	
—	—		
	—	—	

produced significant growth in E. parva. If the mean dry weight of the mycelia grown on these sources is compared with the point averages of Table III we see that the E. parva results are very close to the average of all other fungi studied. A few exceptions to this generalization could be mentioned. Urea and glutamic acid would be expected to support better growth based on data from other fungi.

Upon making a similar comparison between Table VI and Table III we see that the E. crescens results do not correlate as well with the data from other fungi as found in the literature. Serine, glycine, and isoleucine supported better growth than would be expected and arginine and urea supported less growth compared with the literature.

Most fungi utilize ammonium ions better than nitrate ions. Both species of Emmonsia demonstrated this very definitely.

Emmonsia utilizes the aliphatic amino acids and the acidic amino acids well. This genus does not utilize the aromatic amino acids or the sulfur amino acids to any significant extent according to my data. The hydroxyamino acids and the basic amino acids are varied in the extent of their utilization by Emmonsia. The one imino acid tested was a good nitrogen source.

This raises some questions concerning the biochemical relationships of some of these nitrogen sources. The aliphatic amino acids are related biochemically but we find valine not being utilized by either fungus whereas isoleucine is utilized by both species. Of the two hydroxyamino acids tested, one (serine) was utilized by both organisms while threonine was not utilized by either one. Cysteine also has an amino group available but is not utilized as a nitrogen source. Evidently

the utilization of a nitrogen source or lack of such by an organism is due to a specific factor (enzyme) or group of factors which promote transamination of a specific amino acid rather than general factors transaminating all related chemicals.

The analysis conducted in this study raises serious doubts concerning the validity of some of the statements in the mycological literature concerning the significance of nutritional differences. Much of the literature completely ignores statistical analysis. A notable exception to this is Fraser and Fujikawa (1958). Many papers use fewer replicates than I used and some don't give replicate data or variances. When only the mean is stated how can one know whether the conclusions drawn are valid?

My research shows more, perhaps, than the variable utilization of nitrogen sources by the genus *Emmonsia* and the differences between *E. parva* and *E. crescens*. It shows the need for meaningful statistical evaluation of much of the nutritional research which is done in the field of mycology.

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THE NITROGEN NUTRITION OF EMMONSIA (MONILIALES, ALEURISMACEAE)

by

Gary L. Bradley

An Abstract of a Thesis

in Partial Fulfillment of the Requirements

for the Degree Master of Arts

in the Field of Biology

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ABSTRACT

The questions posed in this research concerned both the comparison of the nitrogen nutrition between Emmonsia parva and Emmonsia crescens and the comparative nitrogen utilization within the species. This fungus is a parasite with a rather uncomplicated life-cycle. Thus it lends itself well to study of the host-parasite relationship at the molecular level. This research contributes toward that long-range goal.

Five replicate floating cultures of each species were grown in 125 ml Erlenmeyer flasks containing a chemically defined basal medium plus one of the 29 nitrogen sources tested. Utilization of a particular source was determined by dry weight. The results were quite variable. Where the variances between sources both within species and between species showed relative homogeneity, *t* tests were run.

The data indicates a significant difference in utilization of arginine, alanine, casein, isoleucine, and serine between the two species of Emmonsia. The difference between utilization of ammonium, aspartic acid, and histidine could not be considered significant according to the data. The differences between nitrogen sources within each species is discussed also.

The overwhelming evidence uncovered both in searching the literature and in this research leads to the conclusion that there is a great need for meaningful and accurately applied statistical analysis of nutritional data in the field of mycology.