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Effects of temperature and Ph on the growth and composition of the sporophyte and gametophyte generations of Allomyces arbusculus Butler (1911)

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EFFECTS OF TEMPERATURE AND PH ON THE GROWTH AND COMPOSTITION OF THE SPOROPHYTE AND GAMETOPHYTE GENERATIONS OF *ALLOMYCES ARBUSCULUS* BUTLER (1911)

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ABSTRACT

Pure cultures of the sporophyte and gametophyte generations of Allomyces arbusculus Butler (1911) were grown under varied conditions of temperature and pH. On solid media the gametophyte did not grow at 10 and 15 C; the sporophyte began slow growth after a long lag period at 15 C. There were no significant differences in growth between the sporophyte and gametophyte at 20 and 25 C. Increasing colony areas occurred for both generations at temperatures between 30 and 40 C, with the largest amount of growth for either generation at 35 C.

Colonies grown in broth media at various pH levels produced a larger amount of growth for both generations at all temperatures when the pH was periodically readjusted to the original level during incubation than when there was no readjustment. The optimum pH for both generations with readjusted pH was 7.0; the optimum temperature was 32-37 C. The sporophyte demonstrated a significant difference in the amount of growth as compared to the gametophyte in both readjusted and unadjusted pH conditions.

Chemical analysis of both generations for total nitrogen was done with the Biuret test for protein and total carbohydrates with the anthrone reagent. The differences in the amount

of total protein for both generations proved statistically nonsignificant with a slight increase in protein for both generations at pH 7.0 at temperatures of 27 and 32 C. Results of tests for total carbohydrate for both generations was variable with no definite conclusions being established.

INTRODUCTION

There are few published papers which compare the effects of temperature and pH on growth of the sporophyte and gametophyte generations of Allomyces sp. The object of most research with Allomyces has been the establishment of nutritional conditions which produce the most satisfactory growth of the sporophyte and gametophyte generations.

The genus Allomyces was first described in 1911 by Butler (Hatch and Jones, 1944) but it was not until after Kneip's recognition of the alternation of generations that these organisms were considered as tools for the study of special biological problems (Machlis,1953). The life cycle involving the sporophyte and gametophyte generations was described by Emerson (1941) and Sparrow (1943). Machlis (1953) listed growth factor requirements of Allomyces javanicus and discussed the effects of culture conditions on the maturation of meiosporangia in Euallomyces. The effect on the growth of Allomyces sp. of pH changes and the concentration of nutrients in a minimal synthetic medium was also reported by Machlis (1953). Further investigation by Ingraham and Emerson (1954) reported methionine as an important growth factor in the development of Allomyces arbusculus and A. javanicus. Machlis and Craseman (1956) showed that the sporophyte and gametophyte generations

in the subgenus Euallomyces have the same growth rates in liquid cultures, the same general nutritional requirements and require the same amino acids.

The present investigation was undertaken in an attempt to determine the effect of pH and temperature on growth and protein and carbohydrate content of the sporophyte and gametophyte generations. Although a similar study of some species of Pythium has been done by Cantrell (Cantrell and Dowler, 1971), those fungi produce only haploid vegetative growth. The use of Allomyces makes possible a comparison of diploid (sporophyte) and haploid (gametophyte) vegetative phases of the same organism.

MATERIALS AND METHODS

Experimental Organism

Axenic cultures of Allomyces arbusculus Butler (1911) were obtained from the Carolina Biological Supply Company, Burlington, North Carolina. Pure cultures of both sporophyte and gametophyte were derived from these as described later and were subcultured at 25 C on a solid maintenance medium.

Media

All water used in the experiments was glass-distilled.

Glassware was washed with Alconox and hot water and rinsed twice in tap water and three times with distilled water. For the pH experiments, 125 ml Erlenmeyer flasks were used. Disposable nine cm petri dishes were used for the colony diameter-area experiment.

The basal medium used in all experiments was based on that of Machlis and Ossia (1953) and was prepared as follows:

$MgSO_4 \cdot 7H_2O$	Mallinckrodt's Analytical	0.25 g
KH_2PO_4	Baker's Analyzed	0.01 M
Yeast Extract	Difco Certified	4.00 g
Glucose	Difco Certified	5.00 g
Boric Acid	Elk C P Grade	1/15,000
Distilled Water		1.00 l

The KH_2PO_4 was made up to 0.01 M in a one liter volumetric flask and the other ingredients were added to it. The boric acid at a concentration of 1/15,000 as suggested by Turian (1954) was added to prevent copulation of gametes thus making it possible to maintain pure gametophytic liquid cultures. The pH of the media was tested with a Corning Model 7 pH meter before and after autoclaving. The stock maintenance medium had a pH of 7.0-7.3. This medium was sterilized in the autoclave at 121 C for 15 minutes and dispensed into nine cm disposable petri dishes.

Modifications of the basal medium included the addition of agar (15g/liter) to prepare solid media for maintenance of stock sultures and for Experiment 1. In Experiment 2, pH was adjusted over a range of 6.0 to 7.5 by using sterile 1N HCL or NaOH. In the same experiment, 0.04% bromothymol blue was included in the broth media to serve as pH indicator.

Culture Methods

Two methods were used to obtain axenic stock cultures of the sporophyte. In one of these, male and female gametangia were removed aseptically from plate cultures of the gametophyte and were placed in sterile distilled water for two to four hours. During this period, gametes emerged and fused. The second method was that of Machlis (1953) and involved placing the gametangia in a dilute salt solution instead of distilled water. The salt solution was prepared as follows:

KH_2PO_4	0.001 M
$MgCl_2$	0.001 M
$CaCL_2$	0.00002 M

The pH was adjusted to 7.0 with 1N KOH.

After zygotes had formed, several drops of the suspension were transferred to plates of maintenance agar to initiate pure cultures of the sporophyte.

To prepare inoculum, a sterile cork borer (0.5 cm in diameter) was used to cut discs in the margin of plate cultures The discs were then transferred aseptically to experimental media using a blade fashioned from heavy chromel wire which was flattened and sharpened on one edge to facilitate transfer of inocula.

Cultures were examined frequently during the course of the experiments to verify their purity. The gametophytes had a characteristic orange color and the sporophytes were light brown. Bacterial contamination could be detected by turbidity in the broth media and by the presence of distinct colonies on solid media. Microscopic examination was also used. Contaminated cultures were few and were discarded.

Miscellaneous Equipment and Methods

Coleman buffer tablets were used to prepare standard pH solutions for the spot-plate pH adjustments. These standard solutions contained 0.04% bromothymol blue in the same concentration as the liquid basal medium.

The bovine serum standards for the Biuret test were concentrations of 2, 4, 6, and 8 mg/ml. The glucose standards for the anthrone carbohydrate analysis were in concentrations of 1, 2, 4, 6, and 8 mg/ml. Measurements for the protein and carbohydrate analysis were made with a Bausch and Lomb spectrophotometer.

For the temperature experiments, a refrigerator was used for temperatures below 15 C. The refrigerator temperature was adjusted to the desired level and the thermometer checked daily to insure a relatively constant temperature.

A thermal control room was used to maintain temperatures in the 15 C range and at 20 (± 1 C) C. The control room temperature is regulated by a Kold-Pack refrigerating unit.

Temperatures at 25 C and above were maintained in electrically operated laboratory incubators which controlled the temperatures within 1-2 C.

Experimental

Exp. 1. Effect of Temperature on Growth of Sporophyte and Gametophyte Generations.

The cultures were grown on basal medium plus 1.5% agar at a pH of 7.01.

The temperatures investigated ranged from 10-40 with intervals of five C. There were four replicates each of the sporophyte and gametophyte at each temperature. The cultures were incubated in darkness. Daily measurements of colony diameters were made after the first 24 hours. Total incubation time was 120 hours. Two diameters at right angles were taken for each colony and the average was used to calculate the colony area (Table 1).

Exp. 2. Effect of Temperature and pH on the Growth of Sporophyte and Gametophyte Generations.

Sporophyte and gametophyte cultures were grown under various pH and temperature conditions. The temperature conditions were 27, 32 and 37 C.

The initial pH of the media was adjusted immediately after preparation to include a range from 6.0 to 7.5 with an interval of 0.5 units. The media were dispensed into 125 ml Erlenmeyer flasks and capped with polyurethane sponge stoppers. The media were autoclaved and one flask of each medium was sacrificed for a pH check after sterilization. The media were inoculated with 0.5 cm discs from the margin of stock sporophyte and gametophyte cultures. Triplicate cultures of each stage at each pH were incubated at 27, 32 and 37 C. The flasks containing the pH standards were also incubated with the culture flasks. Following an initial 24 hour period of adjustment to the new substrate, three cultures of sporophyte and three of gametophyte for each experimental condition were checked for pH using the spot-plate method. The pH was then readjusted to the original level with sterile 1N NaOH. The remaining cultures were allowed to incubate without adjustment during the experiment.

A. Media at pH 6.0, 6.5, 7.0 and 7.5 were prepared from basal medium and dispensed at the rate of 50 ml per flask. After 120 hours incubation, the mycelia were harvested by suction filtration through clean cheesecloth, placed on pre-weighed Whatman #1 filter paper in glass petri dishes, and dried at 100 C for 18 hours. The filter paper and mycelia were reweighed at the end of this time period and dry weights for all samples were recorded. The final pH of all media was checked.

B. The media was prepared as in A, and dispensed into flasks at the rate of 75 ml per flask. There were three replicates each of sporophyte and gametophyte for each pH-temperature

condition. After 120 hours, the cultures in which the pH had been adjusted daily were harvested. The mycelia were washed twice with distilled water and refrigerated at approximately 10 C until analysis, prior to which they were washed four more times. The culture filtrate was frozen in sterile 70 ml tubes and stored at -6 C until the pH could be checked. Samples from each of the three replicates of sporophyte and gametophyte at each experimental condition were analyzed for total nitrogen by the Robinson-Hogden biuret method for soluble protein (Norris and Ribbons, 1971). Additional samples from each replicate were analyzed by the anthrone method for total carbohydrate (Norris and Ribbons, 1971).

RESULTS

Colonies grown on solid media were circular; the sporophytes were brown, the gametophytes orange.

Colonies grown in liquid culture varied in total dry weight according to cultural conditions. The total amount of mycelium was larger for controls at all temperatures than those subjected to experimental variations. The color of the control media varied according to pH and indicator; experimental media became a dark yellow due to acid production.

Exp. 1. Effect of Temperature on Growth of Sporophyte and and Gametophyte Generations.

The sporophyte grew at all temperatures except 10 C. The gametophyte grew at all temperatures except 10 and 15 C. At 15 C growth began in the sporophyte after the first 72 hours; limited growth continued at this temperature for the full experimental time. Neither generation grew at 20 C during the first 24 hours. Both generations produced growth after this initial period and final results after 120 hours were similar for both generations. Measurable growth for both generations at 25 C occurred after the first 24 hours with the greatest increase in growth recorded between 96 and 120 hours for both generations. The growth pattern at 30 C was similar, with an increase in colony area for both generations. The largest

amount of growth was recorded at 35 C for both generations; conditions at 40 C produced results which were similar to that of 30 C. There was a slight increase in the total sporophyte area at 40 C as compared to the gametophyte. These results are summarized in Table 1.

A Spearman coefficient of rank correlation (Ferguson, 1971) for colony area at the seven temperature levels was conducted. The correlation analysis revealed that as temperature increased, the area for each generation also increased (Appendix, Table 1).

Exp. 2. Effect of Temperature and pH on Growth of the Sporophyte and Gametophyte Generations.

A. The amount of growth recorded as dry weight varied for sporophyte and gametophyte according to cultural conditions. The largest amount of growth for either generation occurred at pH 7.0 at 37 C for the sporophyte. An analysis of variance of means for mean growth for conditions of temperature, pH and generation in which the pH was readjusted indicated that there were significant differences among the means at the 0.05 level (Appendix, Table 2). A Newman-Keuls test of ordered means (Ferguson, 1971) indicated that growth conditions at pH level 7.0 differed significantly from all other treatments while there was no significant difference between pH levels 6.0 and 6.5 (Appendix, Table 3). The same analysis was made for the temperature levels of 27, 32 and 37 C and indicated a significant difference in the growth at 27 C and growth at 32 and 37 C (Appendix, Table 4). A Newman-Keuls data for the sporophyte and gametophyte generations indicated a significant difference in the total growth, with the sporophyte demonstrating

a significantly larger amount of growth for all conditions (Appendix, Table 5).

An analysis of variance of means was made for the unadjusted pH growth conditions and indicated that a significant difference occurred at the 0.05 level for pH and generation (Appendix, Table 6). A Newman-Keuls test indicated that growth at pH 7.5 was significantly higher than growth at all other pH levels (Appendix, Table 7) and that the sporophyte growth was significantly higher than that of the gametophyte (Appendix, Table 8).

B. Chemical Analysis of Controlled Cultural Conditions of Sporophyte and Gametophyte Generations.

Total nitrogen for both generations varied between 10-20% of the mycelial dry weight. A slight increase in total nitrogen for both generations occurred at pH 7.0 at temperatures 27 and 37 C. Both generations demonstrated a slight decrease in total nitrogen at 32 C for pH 7.0 (Figures 2 and 3). An analysis of variance of mean total nitrogen for conditions of temperature, pH and generation indicated that the differences between all measurements for total nitrogen in the generations were nonsignificant (Appendix, Table 9).

Total carbohydrate for both generations varied between 10-20% of the mycelial dry weight. A decrease in total carbohydrate occurred for both generations at pH 7.0 at temperatures 27 and 37 C; a slight increase in carbohydrate occurred for the sporophyte at 32 C for pH 7.0 (Table 4). An analysis of variance of mean carbohydrate for conditions of temperature, pH and generation was made indicating significance at 0.05

level for all levels of pH and for the interaction between temperature and generation (Appendix, Table 10). A Newman-Keuls test indicated that at pH 7.5 total carbohydrate increased significantly from pH 7.0, but not from 6.0 and 6.5 (Appendix, Table 11). Results from an additional Newman-Keuls test indicated that the sporophyte generation increased significantly in total carbohydrate from the gametophyte at 27 C. At 37 C, the gametophyte increased significantly in total carbohydrate as compared to the sporophyte.

DISCUSSION

No studies directly concerned with the effect of temperature and pH on the comparative growth of the sporophyte and gametophyte generations of Allomyces arbusculus Butler (1911) could be found in the literature. It is recognized that the effects of cultural conditions on organisms vary with specific conditions of time, medium and method of measurement (Cochrane, 1958). In this study, the range of temperatures and pH values simulate some of the conditions to be encountered in the natural environment of the test organism.

For many phycomycetes, aquatic habitats provide relatively stable environments of temperature; pH, however, may fluctuate due to periodic water flow. For a given species, the range for either is a limiting factor for growth. According to Cochrane (1958) most fungi grow over an optimum range of temperatures of 25-30 C. As species of Allomyces have been isolated from areas of temperate and tropical climates, the temperature variation for growth may be quite large. Most species, however, are found in tropical climates which indicates that optimal growth requirements are around 30-35 C.

In Experiment 1 optimal growth for both generations of Allomyces arbusculus Butler (1911) occurred at 35 C (Table 1). Neither sporophyte nor gametophyte grew at 10 C; however, when

these same cultures were transferred to a 30 C incubator, measurable growth was observed for both generations, proving the cultures were still viable. The long lag phase of 72 hours and subsequent slow growth of the sporophyte at 15 C indicate that this temperature is very close to the minimum for that generation. As the gametophyte did not grow at all at 15 C, its minimum must be slightly higher, but less than 20 C. A 0.5% glucose medium was used for this experiment and the results were similar to those of Sistrom and Machlis (1955) who found a lag phase of approximately 100 hours at 25 C in a 0.5% glucose medium.

The greatest amount of growth occurred for both generations at 35 C with the gametophyte producing a slightly larger amount of growth than the sporophyte (Figure 1). Extension of the growth period for the purpose of identifying a linear growth phase was deemed inadvisable, as colonies at 30 C through 40 C grew to within 0.5 to 1.0 cm of the edge of the plate making further growth difficult to measure. Colony diameters were converted to area measurements to give a more reliable account of the total amount of growth.

In Experiment 2 A, growth of both generations was significantly better at 32 and 37 C than at 27 C (Table 2). These results correspond to those in Experiment 1. Where the pH levels were periodically readjusted to their initial value during incubation, growth occurred over the test range, 6.0 to 7.5, but the best growth for both generations was at 7.0 at all temperatures (Table 2). These results agree with those of Emerson and Cantino (1948) who found an optimal growth

range of pH 6.0 to 8.0 with an optimum of 7.0 for sporophytes of A. arbusculus.

In that part of Experiment 2 A in which the pH was not readjusted, values dropped as low as pH 4.70, and total growth at each initial pH was less than in corresponding conditions receiving readjustment (Table 3).

There is some evidence for a difference in growth rate and final amount of growth between the sporophyte and gametophyte in both adjusted and unadjusted pH situations.

The Newman-Keuls analysis indicates a significantly greater amount of growth of the sporophyte as compared to the gametophyte under all conditions. Two exceptions were noted; at pH 7.0 at 27 C with pH readjustment the gametophyte produced a slightly larger amount of growth, and also at pH 7.5 at 27 C with pH unadjusted, the gametophyte produced a slightly larger amount of growth. Machlis and Craseman (1956) stated that the linear growth rates of both generations were the same in liquid culture. However, the conditions of media, temperature and pH for their experiments differed from those used here, making a direct comparison impossible.

The largest amount of growth in unadjusted pH conditions occurred when the initial pH was 7.5. Most fungi produce acidic metabolic by-products that tend to lower the pH as growth proceeds. The nature and rate of production of such by-products depends on a number of factors including the original constituents of the medium, its buffering capacity and the species of fungus. The pH may become the limiting factor for growth before the food supply is exhausted (Lilly and Barnett, 1951).

A relatively high initial pH may have given the organism a favorable environment for a longer period than when the pH was lower at the beginning. Environmental factors such as temperature can produce effects which alter growth at different pH levels (Cochrane, 1958); however, this was not observed in this experiment as temperature and temperature-pH interactions proved to be nonsignificant statistically.

The results of the adjusted and unadjusted pH conditions suggest that with adjusted pH at constant temperature, A. arbusculus can grow until nutrients in the media are exhausted. Emerson and Cantino (1948) demonstrated this by culturing A. arbusculus sporophytes for seven weeks by altering the pH of the media to favorable levels.

The purpose of Experiment 2 B was to determine by chemical analysis if there were any differences in total protein and carbohydrate content between the generations due to pH and/or temperature. Robinson and Hogden (1940) have demonstrated a linear relation between the Kjeldhal procedure for total nitrogen and the biuret test, and the latter method was chosen for this work. Although not all mycelial nitrogen is protein, Cochrane (1958) showed that protein determinations based on the Kjeldhal nitrogen values were directly related to protein in several species of fungi.

Results of the biuret test showed no significant difference in total nitrogen between sporophyte and gametophyte due to temperature, pH or interactions between these factors, except at 32 C where there was a decrease of the gametophyte's total nitrogen. It is interesting to note that at pH 7.0 there

was a slight increase in total nitrogen in both sporophyte and gametophyte generations. The changes in total nitrogen at pH 7.0 suggest that under more favorable growth conditions, the rate of protein synthesis is increased. The data show that the total protein for both generations was between 10-20% of the dry weight. Cochrane (1958) noted that the total protein for fungal mycelia depends on the kind of media, and ranges from 12-38% dry weight in many fungi.

The results of the anthrone test for carbohydrates are not clear. Statistical analysis of the data reveals that pH was significant in affecting the total carbohydrate in both generations. However, the Newman-Keuls analysis of these data does not clearly indicate which pH level is most significant in affecting the total carbohydrate in both generations. An analysis of the temperature-generation data indicates that 27 C was optimal for the sporophyte and 37 C for the gametophyte with no significant difference in the amount of growth for either stage at 32 C. An increase in the carbohydrate content is evident for both generations at pH 7.5 (Table 4). This may reflect an increasing rate of carbohydrate synthesis and storage at a level slightly above the optimum for protein synthesis and growth. These results are subject to question as the mycelia had been stored at 10 C for 33 hours prior to analysis, and under these conditions, some breakdown could have taken place.

In summary, the sporophyte and gametophyte generations of Allomyces arbusculus Baker (1911) seem to demonstrate similar growth characteristics under varied environmental conditions.

However, at temperature conditions of 32-37 C, a larger amount of growth was recorded for the sporophyte than for the gametophyte in Experiment 2 A. The optimal level of pH for both generations was 7.0, a level which is in agreement with values found in the literature and at which the sporophyte produced a larger amount of growth than the gametophyte.

No appreciable difference was recorded in the total protein for either generation, although both showed a slight increase at pH 7.0.

The total carbohydrate content for both generations was between 10 and 20% for the dry weight of the mycelium. Most values cited in the literature for other fungi range from approximately 9 to 60% of the mycelial dry weight.

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Table 1. Mean colony areas in cm^2 of sporophyte and gametophyte generations grown on solid medium.

Temperature	Hour 24 S	24 G	48 S	48 G	72 S	72 G	96 S	96 G	120 S	120 G
10	none		none		none		none		none	
15	none		none		0.41	none	0.41	none	0.74	none
20	none		0.39	0.22	0.72	0.74	1.32	1.72	2.40	2.66
25	0.39	0.58	2.01	3.07	5.10	6.74	9.18	11.34	16.11	15.97
30	1.10	0.81	2.92	4.83	7.64	6.10	14.65	22.30	22.97	22.89
35	0.58	1.56	4.41	6.92	10.17	14.31	19.39	15.13	28.82	32.15
40	0.95	0.83	4.34	4.52	9.24	9.45	20.26	15.19	25.95	22.30

Table 2. Dry weights in mg of sporophyte and gametophyte generations with adjusted pH.

pH	Temperature 27 S	27 G	32 S	32 G	37 S	37 G
6.0	33.3	27.9	40.8	29.8	31.9	25.0
6.5	36.6	31.4	44.2	38.3	45.6	27.9
7.0	43.2	52.4	51.7	46.4	59.4	49.6
7.5	27.9	26.3	44.2	44.2	55.1	37.7

Table 3. Dry weights in mg of sporophyte and gametophyte generations with unadjusted pH.

	Temperature					
	27		32		37	
pH	S	G	S	G	S	G
6.0	12.8	2.1	12.9	5.5	6.1	5.7
6.5	5.7	5.4	7.9	6.9	9.2	7.0
7.0	14.6	14.2	16.2	8.8	8.5	11.5
7.5	18.5	19.7	20.6	11.3	17.0	12.5

Table 4. Mean carbohydrate values for sporophyte and gametophyte generations.

pH	Temperature 27 S	27 G	32 S	32 G	37 S	37 G
6.0	0.196	0.329	0.174	0.143	0.105	0.203
6.5	0.375	0.173	0.082	0.127	0.150	0.195
7.0	0.092	0.039	0.162	0.055	0.056	0.124
7.5	0.364	0.094	0.158	0.082	0.174	0.329

Figure 1. Mean colony areas in cm^2 for sporophyte and gametophyte generations at 35 C.

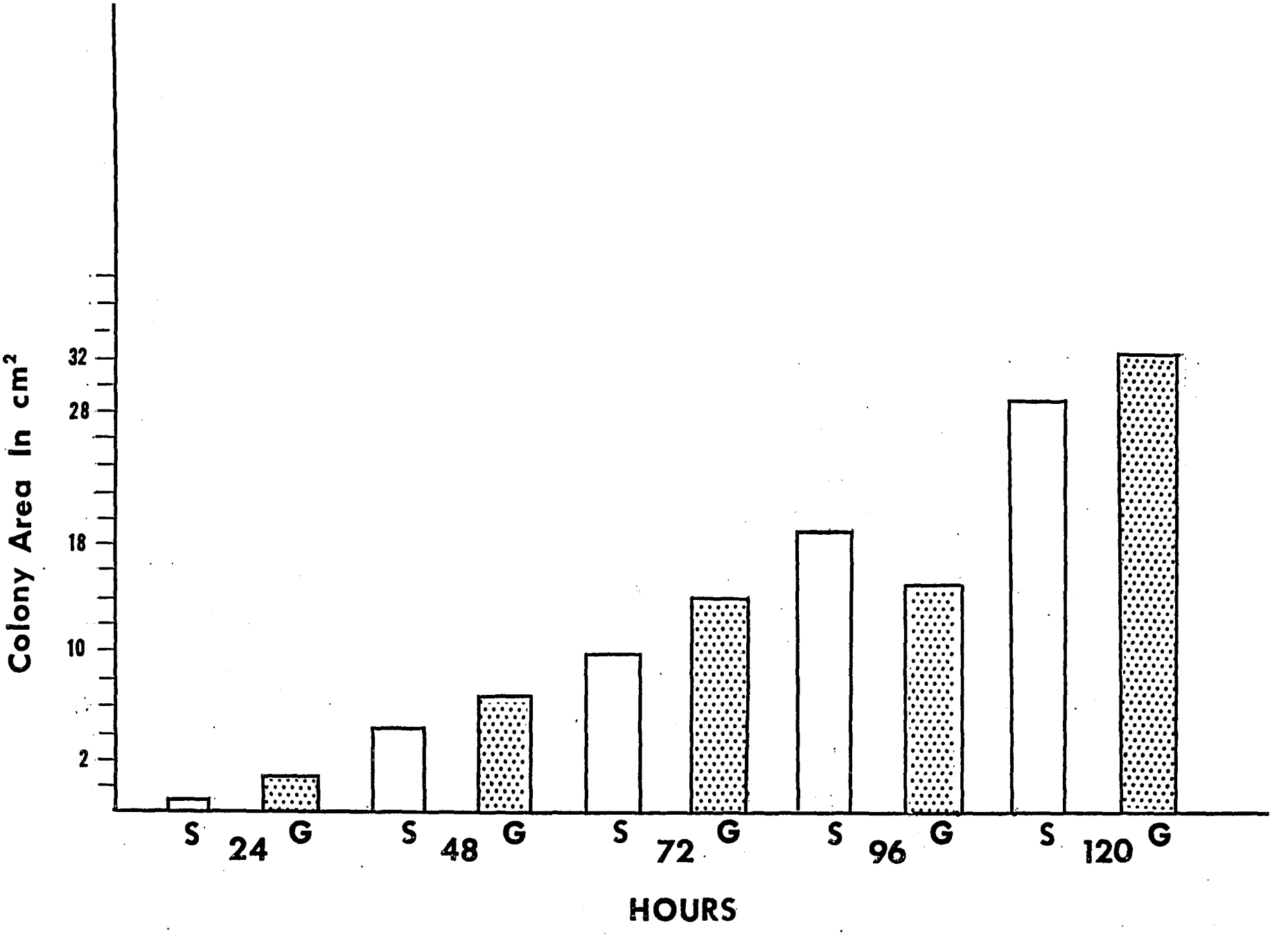

Colony Area In cm²
32
28
18
10
2
S
24
G
S
48
G
S
72
G
S
96
G
S
120
G
HOURS

Figure 2. Mean total protein for sporophyte generation at temperature levels 27, 32 and 37 C with adjusted pH.

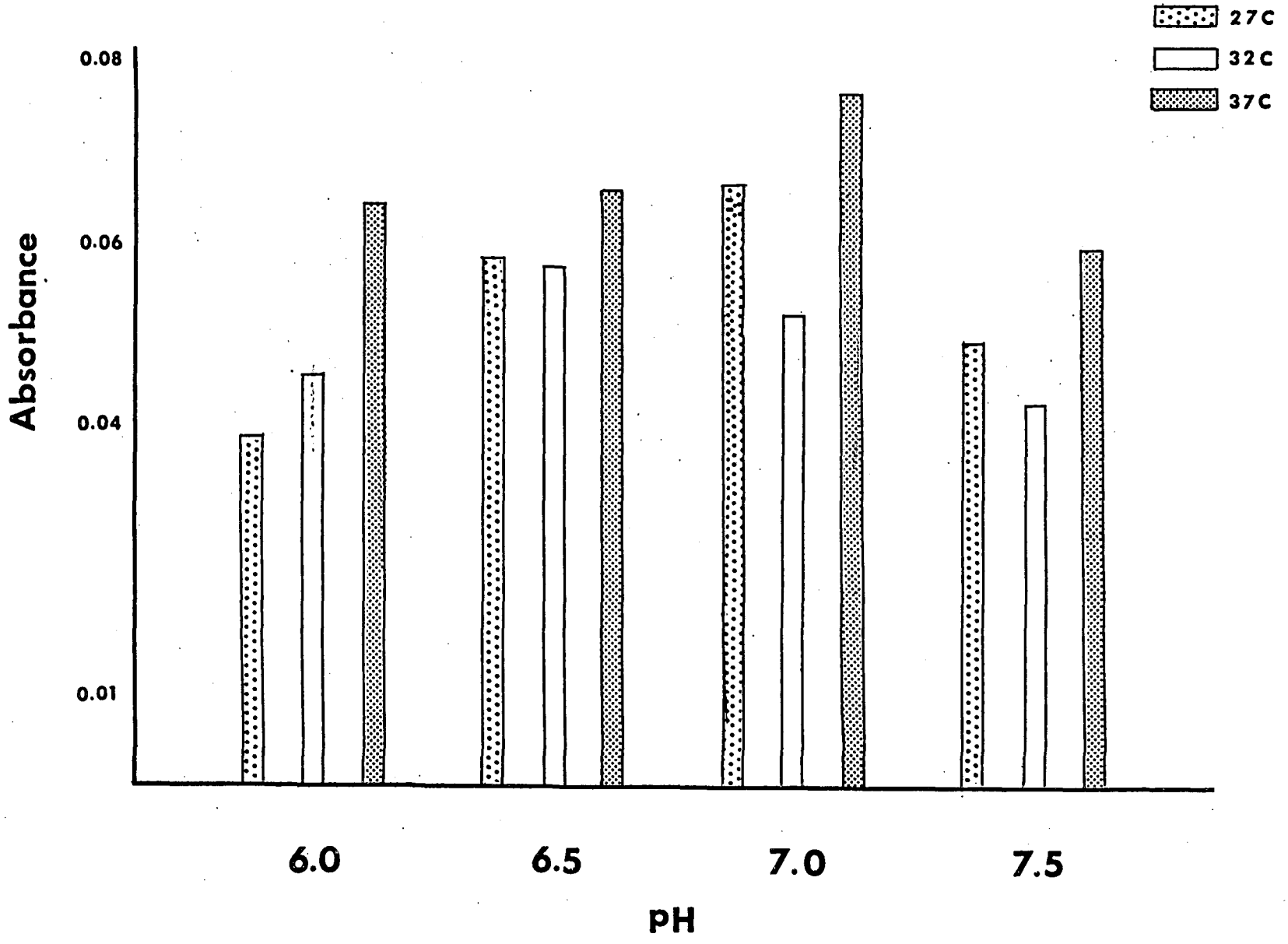
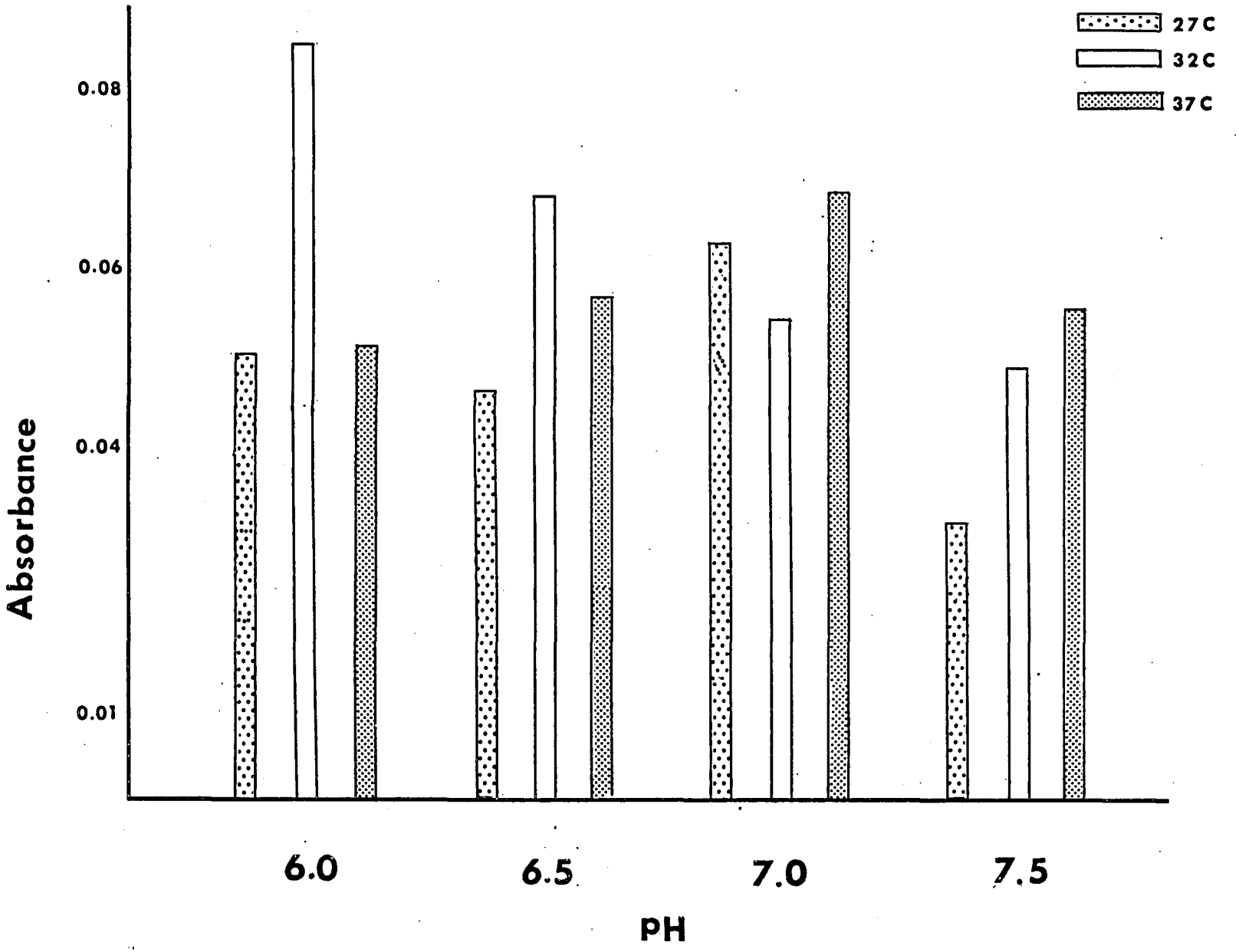

27C
32C
37C
0.08
0.06
0.04
0.01
Absorbance
6.0
6.5
7.0
7.5
pH

Figure 3. Mean total protein for gametophyte generation at temperature levels 27, 32 and 37 C with adjusted pH.


27C
32C
37C
0.08
0.06
0.04
0.01
Absorbance
6.0
6.5
7.0
7.5
PH

APPENDIX

Table 1. Spearman Coefficient of Rank Correlation - Means of colony areas.

Temperature	Rank S	Rank G	Difference d	Difference d^2
10	1	1	0	0
15	2	2	0	0
20	3	3	0	0
25	4	4	0	0
30	5	6	-1	1
35	6	5	1	1
40	7	7	0	0
Total			0	$\sum d^2=2$

$$p = 1-\frac{6(2)}{2394}$$

$$p = 0.9995$$

Table 2. Analysis of Variance - Mean growth values for pH, temperature and generation with readjusted pH.

Source	SS	df	MS	F
pH	3339.83	3	1113.27	13.97*
Temperature	806.06	2	403.03	5.06*
Generation	720.04	1	720.04	9.04*
Error	3742.83	47	79.63	

* significance at the .05 level

$F_{.95}(3,47) = 2.81$
$F_{.95}(2,47) = 3.20$
$F_{.95}(1,47) = 4.04$

Table 3. Newman-Keuls Test of Ordered Means - Mean growth for pH values with readjusted pH.

pH		pH 6.0	6.5	7.5	7.0
	means	31.4	37.3	39.2	50.4
6.0	31.4	--	2.80	3.70*	9.05*
6.5	37.3		--	0.90	6.25*
7.5	39.2			--	5.34*
7.0	50.4				--

* significance at the .05 level

Table 4. Newman-Keuls Test of Ordered Means - Mean temperature growth values for readjusted pH

Temperature		Temperature 27	37	32
	means	34.8	41.5	42.4
27	34.8	--	3.65*	4.16*
37	41.5		--	0.51
32	42.4			--

*significance at the .05 level

Table 5. Newman-Keuls Test of Ordered Means - Mean generation growth for readjusted pH.

Generation	means	Generation gametophyte 36.41	Generation sporophyte 42.84
gametophyte	36.41	--	4.32*
sporophyte	42.84		--

Table 6. Analysis of Variance - Mean growth values for pH, temperature and generation with unadjusted pH.

Source	SS	df	MS	F
pH	1097.43	3	365.67	21.75*
Temperature	46.58	2	23.92	1.38
Generation	195.59	1	195.59	11.63*
Error	789.93	47	16.80	

* significance at the .05 level

$F_{.95}(3,47) = 2.81$
$F_{.95}(1,47) = 4.05$

Table 7. Newman-Keuls Test of Ordered Means - Mean growth for pH values with unadjusted pH.

pH		pH 6.5	pH 6.0	pH 7.0	pH 7.5
	means	7.02	7.48	12.30	16.60
6.5	7.02	--	0.48	5.50*	9.98*
6.0	7.48		--	5.02*	9.50*
7.0	12.30			--	4.48*
7.5	16.60				--

* significance at the .05 level

Table 8. Newman-Keuls Test of Ordered Means - Mean generation growth for unadjusted pH.

Generation		Generation	
		gametophyte	sporophyte
Generation	means	9.23	12.23
gametophyte	9.23	--	4.81*
sporophyte	12.50		--

* significance at the .05 level

Table 9. Analysis of Variance - Mean nitrogen values.

Source	SS	df	MS	F
pH	0.002	3	0.000	2.05
Temperature	0.001	2	0.000	2.75
Generation	0.000	1	0.000	0.46
Error	0.015	46	0.000	

Table 10. Analysis of Variance - Mean carbohydrate values.

Source	SS	df	MS	F
pH	0.166	3	0.055	4.32*
Temperature	0.076	2	0.038	2.99
Generation	0.001	1	0.001	0.125
Temperature-Generation	0.091	2	0.045	3.55*
Error	0.577	45	0.012	

* significance at the .05 level $F_{.95}(3,45) = 2.82$
$F_{.95}(2,45) = 3.21$

Table 11. Newman-Keuls Test of Ordered Means - Mean carbohydrate content for pH values for anthrone test.

pH		pH 7.0	6.5	6.0	7.5
	means	0.08	0.18	0.19	0.20
7.0	0.08	--	10.0*	11.0*	12.0*
6.5	0.18		--	1.0	2.0
6.0	0.19			--	1.0
7.5	0.20				--

* significance at the .05 level

VITA

Lawrence Wayne Lenz was born in Washington, D.C. on December 17, 1946. He received his secondary education from Victoria High School, Victoria, Virginia from which he was graduated in June, 1965. He entered Roanoke College in September, 1965 and while there elected to membership in Beta Beta Beta Honorary Biological Society in October, 1967. While at Roanoke College his interest in fungi developed through a course taught by Dr. Phillip C. Lee. In June, 1969 he received the B.S. degree from Roanoke College and accepted a teaching position with the City of Petersburg Public Schools. He was accepted for graduate studies at the University of Richmond and entered in the fall of 1971. While at the University he was awarded the Williams Graduate Fellowship for the second semester 1972 and 1972-73 session. He received his Master of Science degree from the University of Richmond in May, 1973. His future plans are uncertain.