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SOME EFFECTS OF pH, TEMPERATURE AND LIGHT ON THE PRODUCTION OF ZOOSPORANGIA IN *SAPROLEGNIA PARASITICA* COKER

by

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SOME EFFECTS OF pH, TEMPERATURE AND LIGHT ON THE PRODUCTION OF ZOOSPORANGIA IN SAPROLEGNIA PARASITICA COKER

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ABSTRACT

Pure cultures of Saprolegnia parasitica Coker were grown under varied conditions of temperature, pH, and light conditions. In liquid medium colonies were spherical with a zonal effect in the distribution of zoosporangia. Near the limiting extremes of pH and temperature, beaded hyphal tips were found in place of normal sporangia. Teorell Universal buffer in the sporulation water inhibited production of sporangia. The pH minimum was 4.0 - 4.1, optimum 6.3 - 7.4, and maximum 8.0 - 8.3. The maximum number of sporangia was produced at 15 C. Between 3.4 and 5 C, a delay in sporulation was noted and at 0 - 1 C, no sporangia were formed. At 30 C and above, only abnormal sporangia formed.

Cultures grown in continuous artificial light produced double the number of sporangia compared with colonies grown in complete darkness. The average diameters of the colonies grown in complete darkness were 25 % less than average diameters of colonies grown in complete light. When cultures were grown in alternating light and darkness, those that received the greatest total illumination produced the greatest numbers of sporangia.

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INTRODUCTION

There are few published reports dealing specifically with the effects of environmental conditions on zoosporangium production in Saprolegnia parasitica Coker. A number of authors have studied the class Phycomycetes in general.

The temperature factor has been studied most intensely. Klebs (1900) studied the effects of temperature on zoospore liberation in Saprolegnia mixta de Bary. Cotner (1930) surveyed a number of Phycomycetes and listed the temperature optima for sporulation. Weston (1941) gave the temperature optima and extremes for growth and sporulation of Saprolegnia parasitica as stated by Tiffney, whose paper was unavailable for direct consultation. Cardinal temperatures and the influence of light were reported by Duff (1929) for an unidentified species of Saprolegnia, and Coker (1923) worked with the effect of temperature on zoosporulation of S. anisospora de Bary. Lilly and Barnett (1951) and Christenberry (1938) showed the influence of light conditions on the formation of sporangia by Choanephora cucurbitarum. According to Hawker (1957), light may stimulate sporulation in some fungi and inhibit it in others. Weston (1941), reported that Lund found many species of Phycomycetes in natural waters over a wide pH range, and described their pH requirements under laboratory conditions.

The physiological influences of pH, light and temperature on zoosporulation of S. parasitica have been little studied. This thesis was undertaken as an attempt to illucidate some of the influences of these factors.

MATERIALS AND METHODS

The organism used in all experiments was Saprolegnia parasitica Coker, American Type Culture Collection number 11393. It was subcultured by asexual means, either from hyphal tip or zoospore inoculations. Stock cultures were freed from bacterial contamination by the glass-ring method suggested by Smith (1950). The contaminated mycelium was placed inside a sterile van Tiegham ring on the center of a plate of pea agar. Bacterial growth was confined within the ring, while the fungus mycelium grew under the ring and emerged uncontaminated. Other methods that were tried and found to be ineffective were those described by Chaze (1933), and Blank and Tiffney (1936). The few contaminated cultures found in the experiments were discarded.

Media:

All water used in preparing media and in inducing sporulation was doubly distilled, first through a copper still and then through a glass still.

Pea agar was prepared as follows: One hundred dried peas were boiled in distilled water for one-half hour. The resulting broth was strained through cheese cloth, diluted to one liter, and 15 grams of agar were added (Pieters, 1915).

The medium was autoclaved at 15 pounds for 15 minutes and dispensed aseptically into sterile Petri dishes. The plates were stored in a refrigerator until used.

A medium described by Reischer (1951) was used as the basal medium for all experiments, and was prepared as follows:

Glucose	(Baker's Analyzed)	5.0 g
Soluble Starch	(Baker's Analyzed)	5.0 g
Yeast Extract	(Difco)	1.0 g
Doubly distilled water		1.0 l

The dry ingredients were added to the water and dissolved by boiling for one minute and the medium was sterilized in the autoclave at 15 pounds for 15 minutes. The pH of the autoclaved medium was 6.2 - 6.4. The medium was used without modification in the light experiments and in the temperature experiments. In pH experiments, the pH was adjusted with HCl and NaOH, or with Teorell Universal Buffer, as described later. Coleman buffer tablets were used to prepare standards for the spot-plate colorimetric method used to adjust the pH to approximately the desired level before autoclaving. After autoclaving, pH determinations were made with the Coleman Model D-3 pH meter.

A non-nutrient medium made of distilled water with 1.5 % Difco agar was used in preparing inoculum.

Culture Methods:

Single-spore inoculum was used throughout the experimental work. Stock cultures were allowed to sporulate in sterile distilled water at a pH of 6.5. Approximately 0.5 ml of this spore-containing water was distributed over the surface of a water-agar plate. When microscopic examination showed that the spores had germinated, (usually after five to ten hours), the germinated spores were removed singly and used to inoculate the experimental medium. For this purpose, a special blade was prepared from a piece of Chromel-A wire, of the type used for inoculating loops. The end of the wire was flattened and then ground to form a fine blade. Isolated, germinated spores were located under the microscope and the blade was used to cut out a cube of agar containing the spore, which was then transferred to the culture dish.

Standard nine cm Petri dishes were used as culture dishes. They were cleaned with chromic acid solution and rinsed first with tap water and then with distilled water before sterilization in a hot-air oven.

All cultures were grown in Reischer's liquid medium to produce vegetative growth, and then transferred to 50 ml of sterile, doubly distilled water for sporulation. The period of vegetative growth is referred to hereafter as the "presporulation" period, and the period in distilled water is referred to as the "sporulation" period.

Zoosporangium counts:

Following the sporulation period, the water was pipetted from the sporulation dish, and a gentle stream of water from a pipette was directed over the mycelium in such a manner as to spread the fungus growth out evenly in the bottom of the dish. The culture was then carefully drained. If a number of cultures were to be counted, all were killed with osmic acid before draining, to prevent the formation of additional zoosporangia or discharge of zoospores from those sporangia present. The drained mycelium remained in the bottom half of the Petri dish for microscopic examination and zoosporangium counts. Staining of sporangia as suggested by Couch (1939) was not necessary with proper adjustment of light from the sub-stage mirror.

All counts were made at a magnification of 30 X, using a binocular dissecting microscope. A micrometer disc counting grid, 5 mm square and divided into 25, 1-mm squares, was calibrated with a stage micrometer. The entire field of view within the grid was 14.82 sq mm. (Fig. 1).

The Petri dish containing the drained mycelium was placed on the stage of the microscope so that the first area of count was delimited by the grid of the micrometer disc. All zoosporangia in a linear series of adjacent areas, extending across the diameter of the mycelium were counted. The number of areas per mycelium varied with the diameter of the mycelium. A hand tally counter was used to register the number of zoosporangia counted.

pH Experiments:

The pH experiments were the first experiments in this study and several methods of counting zoosporangia were tried. Counts were made in the usual manner with the exception of experiment 4, where the culture was moved on the microscope stage so that four areas were counted on each culture. These areas formed a cross and covered the zone of the mycelium that contained the greatest number of zoosporangia. Thus, higher numerical averages were found in this experiment.

Several buffers were investigated and the Teorell buffer was chosen as all organic constituents were at the same concentration at all pH values; the difference in pH was determined by the amount of 0.1 N NCl added. To keep experimental conditions standard, it was necessary to have the percentage of constituents of the simple medium identical to that of the buffered medium. As the addition of buffer to the simple medium would have diluted it, a modification was used in preparing the simple medium. A stock solution was prepared by dissolving 5.0 g of glucose, 5.0 g of soluble starch, and 1.0 g of yeast extract in cold water; the water level was then brought up to 100 ml. Ten ml of stock solution were diluted to 100 ml, which contained the same percentage of constituents as did the regularly prepared medium. In this way, buffer solutions could be substituted for dilution water, resulting in the standard medium at any desired pH level. The concentrated stock medium, buffer stock

solution, HCl and water were autoclaved separately and mixed under aseptic conditions.

The pH conditions investigated were as follows; (results are recorded in tables numbered to correspond with experiment number.)

Exp. 1 - pH adjusted with HCl and NaOH. Cultures were grown for 35.5 hours at varied pH levels and allowed to sporulate for 24 hours in water of pH 3.0.

Exp. 2 - pH adjusted with HCl and NaOH. Cultures were grown for 47 hours at varied pH and allowed to sporulate for 24.5 hours in water or corresponding pH.

Exp. 3 - pH adjusted with HCl and NaOH. Cultures were grown for 24 hours at pH of 6.4 and allowed to sporulate for 24 hours in water of varied pH.

Exp. 4 - pH of sporulation water adjusted with buffer. Cultures were grown for 35 hours at pH of 6.4 and allowed to sporulate for 26 hours in water adjusted with full-strength buffer.

Exp. 5 - pH of water adjusted with buffer. Cultures were grown for 40 hours at pH of 6.5 and allowed to sporulate for 26 hours in water that was adjusted with full-strength buffer.

Exp. 6 - pH of water adjusted with buffer. Cultures were grown for 34 hours at pH of 6.4 and allowed to sporulate for 24 hours in 1:1, water-buffer solution.

Exp. 7 - pH of medium adjusted with buffer, water pH not adjusted. Cultures were grown for 41 hours at varied pH and allowed to sporulate for 24 hours in plain water of pH 6.9.

Exp. 8 - pH of medium and water adjusted with buffer. Cultures were grown for 41 hours in buffered medium at varied pH levels. Sporulation period was for 24 hours in 1:1, water-buffer solution.

Temperature Experiments:

Refrigerators were used for cultures incubated at temperatures below 15 C. The refrigerator temperature was adjusted to the desired level; a maximum-minimum thermometer was placed inside the refrigerator with the cultures during the experiments.

A water-cooled incubator was improvised to maintain temperatures in the 15 C range. A fruit-juice can (11 cm in diameter and 16 cm deep), containing the cultures, was placed inside a crock (16 cm in diameter and 16 cm deep). Rubber stoppers were used to wedge the can firmly in place within the crock, leaving a space of 2.5 cm between the sides and bottom of the can and the inner wall of the crock. The assembly was covered with an eartherware lid, and water was conducted into the space surrounding the inner container, through a rubber hose inserted under the lid. To further insulate the unit, it was placed inside a second, larger

crock which was in turn placed inside a water-filled, metal tub. The complete assembly was placed in a laboratory sink for drainage. After several hours of water circulation, the temperature inside the unit reached a constant 15 C. Cultures in Petri plates were stacked inside the inner container, and a short thermometer was placed on top of the plates. Frequent checks of the thermometer during a trial period showed no change in temperature when the unit was in operation. The temperature was checked at the beginning and end of experiments.

Basement laboratory rooms were used to maintain temperatures in the 22.2 C and 24 C ranges. The maximum-minimum thermometer was used to check temperature fluctuations, which did not vary over 2 C. Temperatures above 25 C were maintained in electrically-operated laboratory incubators, which controlled the temperature within 2 degrees C.
The temperature conditions investigated were:

Exp. 9 - Presporulation constant 24 C (25 hours), sporulation at varied temperatures (25 hours).

Exp. 10 - Presporulation temperature constant 20 - 21 C (40½ hours), sporulation at varied high and low temperatures (24 hours).

Exp. 11 - Presporulation and sporulation temperatures varied.

Light Experiments:

A basement photographic darkroom was used as the laboratory for this series of experiments. Cultures grown in continuous light or alternating light and darkness were illuminated by two standard white 20 watt fluorescent tubes, 58 cm in length. The tubes were installed in a standard ceiling-type fixture that was placed across the open top of the incubation box. The box was made of wood and was 50 cm long x 30 cm wide x 28 cm deep. Culture plates were arranged on the floor of the box, 26 cm from the light source.

Cultures grown in darkness were incubated inside light-tight, closed table drawers that were 54 cm long x 43 cm wide x 7 cm deep.

An effort was made to maintain uniform light conditions during the transfer of cultures to sporulation water. Cultures from light series were held approximately 26 cm from the light source during transfer and those from darkness series were transferred in the illumination provided by a photographic safe-light equipped with a Wratten Series 0A filter. Temperature fluctuations in all experiments were determined with a maximum-minimum thermometer located in the immediate vicinity of the cultures.

The light conditions investigated were:

Exp. 12 - Growth in continuous light (38 hours), sporulation in continuous light (24 hours), at a constant temperature of 24.4 C for both phases.

Exp. 13 - Growth in continuous darkness (38½ hours), sporulation in continuous darkness (25½ hours) at a constant temperature of 20 C for both phases.

Exp. 14 - Growth in light (28 hours) followed by darkness (10½ hours); sporulation in darkness (10½ hours) followed by light (16 hours). Temperature in light phase was 22.5 C; in darkness 21.1 C.

Exp. 15 - Growth in darkness (38 hours) followed by light (10½ hours); sporulation in light (10½ hours) followed by darkness (16 hours). Temperature in light was 22.5 C; in darkness, 21.1 C.

Exp. 16 - Growth in light (18 hours) followed by darkness (24½ hours); sporulation in darkness (25 hours). Temperature in light was 24.4 C; in darkness, 21.1 C.

Exp. 17 - Growth in light (18 hours) followed by darkness (24½ hours); sporulation in light (25 hours). Temperature in light was 24.4 C; in darkness, 21.1 C.

Exp. 18 - Growth in light (18 hours) followed by darkness (24½ hours); sporulation in light (25 hours). Temperature in light was 24.4 C; in darkness, 21.1 C.

Exp. 19 - Growth in darkness (18 hours), followed by light (24½ hours); sporulation in light (25 hours). Temperature in light was 22.8 C; in darkness, 21.1 C.

RESULTS

Colonies grown in liquid culture were spherical, with a heavy zone of sporangia beginning approximately 5 - 10 mm from the center of the colony and ending approximately 2 - 3 mm from the periphery. Sporulation in the center of the colony was sparse, and in most cases, no sporangia formed at the periphery until after the first 12 hours of sporulation (Fig. 2).

In pure-culture techniques where pea agar was used, the mycelium reached a diameter of 7 cm after about 24 hours growth and the peripheral zone of approximately 8 mm width showed only vegetative hyphae. Sporangia formed profusely throughout the central area of the mycelium on all pea agar plates, but sporulation was not observed on nutrient agar or plain agar plates.

Although most of the growth of the mycelium occurred during the presporulation period, some additional increase took place after the cultures were transferred to sporulation water.

pH:

Although this paper is primarily concerned with the sporulation of Saprolegnia parasitica, it is of interest to note some effects of the factors on vegetative growth. The lowest pH for growth was 4.8 (Table 2), the highest 8.3 (Tables 7 and 8), and the optimum was approximately 6.5 - 7.4 (Tables 2 and 4).

When the pH of the presporulation medium was adjusted with HCl and NaOH, good growth, but only a small amount of sporulation occurred at pH 5.4 (Table 2). The greatest number of sporangia was produced at a presporulation pH of 6.3 - 7.4 (Tables 2 and 4) and sporulation declined at pH 7.1 (Table 2), in the experiment where presporulation and sporulation pH were varied to the same degree.

In experiment 5, full-strength buffer solution was used for sporulation water; in experiment 6, water-buffer solution 1:1, was used. Tables 5 and 6 show that more sporangia were formed when the buffer concentration in the sporulation water was lower (cultures in Table 5 which show fewer sporangia than cultures in Table 6, were grown for a 6-hour longer period than were cultures in Table 6).

In experiment 7, the buffer was incorporated into the medium and plain water was used for sporulation. A sharp decline in number of sporangia was shown in cultures 4 and 5 of the experiment.

In experiment 8, the buffer was incorporated into the medium and sporulation water. No sporangia were formed in this experiment, but at pH 5.6, hyphal tips were formed in which the cytoplasm was beaded (Table 8).

The lowest pH at which sporangia were formed was 4.0 - 4.1 (Tables 4 and 6). In the cultures adjusted with HCl and NaOH, the largest number of sporangia was produced in the 6.4 - 6.8 range, and in the buffered series, the largest number was found in the range 6.8 to 7.4 (Table 2).

Above pH 8.0, beaded hyphal tips were found in most instances (Tables 2, 5, 6 and 8).

Temperature:

In the temperature experiments, two approaches were used. In experiment 9, the sporulation temperature alone was varied and in experiments 10 and 11, both presporulation and sporulation temperatures were varied. The pH of the medium and water was maintained at approximately 6.4 for all cultures. In general cultures sporulated above the 30 C range contained motile zoospores, although typical sporangia were not present. No zoosporangia were observed in cultures placed in sporulation water at a temperature of one degree C or below (Tables 11 and 12).

Light:

Cultures grown and sporulated in light (Table 12), produced more than twice the number of sporangia as compared to those grown and sporulated in complete darkness. In light, there was approximately 25 % more growth as determined by mycelium diameter comparison. (Tables 12 and 13).

When the fungus was grown and sporulated under conditions of alternating light and darkness, the cultures that received more illumination produced more sporangia. Beaded hyphal tips were not observed in any of the light experiments.

DISCUSSION

No studies directly concerned with pH effects on zoosporangium production in S. parasitica could be found in the literature. Weston (1941) stated that the Phycomycetes in general exhibit a wide range of pH tolerance in natural waters, and that S. delica grew better in highly acidic water. Weston cited maximum, minimum and optimum values for sporulation of S. parasitica, as found by W. N. Tiffney. In a letter to this author, Tiffney stated that he did not write the paper in question, so the figures quoted by Weston could not be verified. Cochrane (1958) mentioned the widely accepted principle that fungi generally grow vegetatively over a wider range than that in which they will sporulate. Spore germination, in turn, takes place in an even narrower pH range.

In this study, maintaining pH of media at the desired level after adjustment proved a major problem. Media tended to change pH upon autoclaving and upon storage. Stock media which was adjusted to basicity with NaOH, dropped as much as one unit within an hour or two after autoclaving, and as much as two pH units after 24 hours. This change was probably due to absorption of CO_2 from the atmosphere. Media adjusted to acidity with HCl did not change appreciably after autoclaving or standing overnight. As externally induced pH changes would have been misleading, all media were allowed to stand 24 hours before use, and only those that had not changed more than 0.2 pH units were inoculated.

Changes that occurred during the relatively short incubation periods were assumed to be due to metabolic activities of the fungus (Tables 1 - 8).

Tables 2 and 4 indicate the optimum pH range for zoosporangium production of S. parasitica (6.3 - 7.4) was almost the same as that for vegetative growth (6.5 - 7.4). The results did not warrant a more exact estimate than that expressed by these ranges. The minimum for zoosporangium production was 4.1; the maximum, 8.0. Weston (1941), citing Tiffney, gave the optimum for zoospore production in S. parasitica as 7 - 8, the minimum as 4.0, and the maximum was 8.2. The figures for the maximum and minimum agreed well with those found in this study; those for the optimum were somewhat higher.

At pH values approaching the maximum and minimum for sporulation, abnormal hyphal tips were observed. Some of these structures resembled gemmae, which, according to Berkeley (1944) give rise to zoospores. A condition best described as "beaded hyphal tips" was also noted (Tables 4, 5, 8 and 10); no reference to this condition has been found in the literature. At 30 X magnification, these hyphal tips appeared to contain large beads of cytoplasm; at 970 X the beaded appearance was not evident, and the hyphal tips were seen to contain regions where the cytoplasm was tightly pressed to the cell wall. Scattered lumps of cytoplasm protruded into the lumen of the hypha, but did not extend completely across (Fig. 3). The lumps contained large granules that resembled nuclei, but the actual nature

of the granules was not determined. No septum was present at the base of the beaded hyphal tips, nor were the tips swollen as usually found in sporangia and gemmae. Beaded hyphal tips were never seen to produce zoospores. Beaded tips were also observed in temperature experiments where conditions were near limiting, and in increasing numbers in cultures older than 48 hours.

Maxima, minima and optima pH values were essentially the same when the fungus was grown under various conditions, although the actual number of sporangia produced at the cardinal values varied considerably from one set of conditions to the next (Fig. 4). The greatest numbers of sporangia were produced where HCl and NaOH were used to adjust pH (Table 2). Teorell buffer suppressed the actual number of sporangia produced, although the cardinal values were comparable to those where HCl and NaOH were used (Tables 7 and 8). Higher concentrations of buffer yielded smaller numbers of sporangia, or completely suppressed sporulation. Buffer produced greater suppression of sporulation when included only in the sporulation water than when included only in the presporulation medium (Tables 5 and 7). The Teorell buffer contains citric acid, which is used by some fungi as a carbon source (Cochrane, 1958). Saprolegnia parasitica sporulates best when no organic materials are present, and it is possible that suppression of sporulation is due to the citrate present in the buffer. Further work is indicated to test this hypothesis.

Zoospores incubated at 0 - 1 C for periods up to two weeks produced no growth and therefore no sporulation. After 5 days at 3.4 - 5 C, zoospores produced branched germ tubes approximately 1.5 mm in length; beaded hyphal tips developed on the branches, but sporangia did not. No close determination of the optimum temperature for growth was attempted in this study; Weston (1941) reported that Tiffney found vigorous growth of S. parasitica at 10 C; growth increased with temperature until approximately twice as rapid at 25 C as at 10 C. In the present study the lowest temperature at which sporangia were produced was 8 C, the highest was 24 C, with few sporangia and beaded hyphal tips between 25 - 30 C. Beaded hyphal tips were formed at 30 C and 34.5 C, but were absent at 36 C. The optimum temperature for sporulation was 15 C (Fig. 5); these figures agree closely with those reported by Weston. Klebs (1900) generalized that the temperature limits permitting sporulation of fungi were narrower than those permitting vegetative growth. Saprolegnia mixta was one of the fungi studied by Klebs in the series of experiments leading to that generalization. Saprolegnia parasitica conforms to Klebs generalization as growth of cultures took place at higher and lower temperatures than did sporulation (Tables 10 and 11).

It is known that light may have a profound influence upon growth and sporulation of some fungi, and may not influence others. Lilly and Barnett (1951) and Christenberry (1938) have shown that Choanephora cucurbitarum,

(a Phycomycete), is extremely sensitive to light conditions in its asexual sporulation. Many other examples of light influences are given by Lilly and Barnett (1951) and Chchrane (1958). Cochrane (1958) recognized two types of fungi with respect to the influence of light upon their sporulation: Those that sporulate well in darkness but poorly or not at all in light; and those that sporulate in light but poorly or not at all in darkness. Duff (1929) showed that ordinary sunlight through glass had no effect on growth of an unidentified species of Saprolegnia: no effect on sporulation was mentioned. No report other than Duff's has been found, dealing with a member of the genus Saprolegnia.

Saprolegnia parasitica seems to fall between the two extremes of fungi as recognized by Cochrane (1958). Sporangia were produced both in complete darkness and in continuous light, but the number of sporangia produced in light was approximately double that produced in darkness (Tables 12 and 13). The increased sporulation under light conditions occurred in spite of a 4.4 C temperature difference, which, from results of temperature experiments, should have had a suppressing effect on sporangial development. This suggests an interrelationship between the effects of light and temperature. Such relationships are known to exist in other fungi (Lilly and Barnett, 1951).

Similar experiments, in which less difference (2.5 C) in temperature was permitted between the light and darkness, showed an even greater difference in sporulation in favor

of the cultures grown in light. Cultures grown and sporulated under varying light conditions did not always give clear-cut results. In general the trend of the experiments involving alternating light and darkness showed that increasing the total amount of light during growth and sporulation increased the number of sporangia produced. The increase took place regardless of the order in which illumination and darkness occurred.

In addition to the observations concerned specifically with pH, temperature and light, some other phenomena of general interest were noted. In all conditions where sporulation occurred, the sporangia were produced in a zone between the periphery and center of the colony. No explanation is offered for this phenomenon. Cochrane (1958) stated that many factors may be responsible for zonation of reproductive structures, depending upon the nature of the fungus. In some cases, it has been shown to be caused by light, in other cases by temperature, and in still others, by nutritional factors. The experiments here indicate that neither light, temperature nor pH was responsible.

Increase in growth was observed after the cultures were transferred from nutrient medium to sporulation water, in spite of the fact that the mycelium was thoroughly rinsed before transfer. Such growth probably resulted from the utilization of stored food and from water uptake.

SUMMARY

Saprolegnia parasitica Coker was grown in pure culture under controlled conditions of pH, temperature and light. Investigations of pH were made using HCl and NaOH, and the Teorell Universal Buffer to adjust pH levels. During temperature studies, the sporulation temperature alone was varied in one series of experiments; the presporulation and sporulation temperatures were varied in another series. In light experiments, series of cultures were grown in full light, complete darkness, and varied combinations of light and darkness.

1. Near the extremes of pH and temperature conditions, beaded hyphal tips were found in place of normal sporangia. No beaded hyphal tips were found in light-darkness experiments.
2. In the pH studies it was found that increased concentration of the buffer solution tended to decrease production of sporangia. This may indicate that organic constituents of the buffer supplied nutrients.
3. The lowest pH where sporangia were found was 4.0 - 4.1. At pH 5.4, good vegetative growth was seen, but few sporangia formed. The greatest number of sporangia was produced in the 6.3 - 7.4 pH range. Above pH 8.0, few normal sporangia formed.
4. No sporangia were formed at temperatures 0 - 1 C, during the normal 25 hour sporulation period, nor after being held at these temperatures for more than one week. At temperatures 3.4 - 5 C, cultures sporulated slowly and at

8 C, zoosporangia were found in all cultures. The greatest number of zoosporangia was produced at 15 C, and above 30 C, few sporangia were formed.

5. Cultures grown in constant light produced almost twice as many sporangia as did cultures grown in constant darkness. There was approximately 25 % greater mycelium diameter in cultures grown in complete light than in complete darkness.

6. Results of growth studies in alternating light and darkness indicated that the total amount of light received was the determining factor in sporulation. Cultures that received the greatest amount of light were the ones that generally showed the greatest number of zoosporangia.

7. When grown in liquid medium, the spherical mycelium of S. parasitica showed a zonal pattern of zoosporangia. Sporangia tended to be more abundant midway between the center and surface of the sphere.

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TABLE 1

Presporulation pH varied; sporulation pH 3.0.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter (mm)	Average Count
3.0	3.2	no growth		-	-
5.1	5.3	3.0	3.1	17	0
6.8	5.5	3.0	3.2	15	0
10.3	9.2	no growth		-	-

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TABLE 2

Presporulation and sporulation pH varied to same degree.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter	Average Count
3.4	3.4	no growth		-	-
4.3	4.3	no growth		-	-
5.4	5.3	5.3	6.1	28	87
6.3	6.1	6.4	6.8	28	164
7.1	6.9	7.1	7.1	34	114
7.4	6.7	7.5	6.9	39	117
8.3	7.8	8.1	7.2	25	101

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TABLE 3

Presporulation pH 6.4, sporulation pH varied.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter	Average Count
6.4	6.3	3.5	3.5	10	0
6.4	5.9	4.1	3.8	10	0
6.4	5.4	6.5	6.7	10	62
6.4	6.5	7.4	7.2	9	59
6.4	6.3	9.2	7.4	12	0

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TABLE 4

Presporulation pH constant 6.4. Sporulation pH varied with full-strength buffer. Special counting method used in this experiment.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter	Average Count
6.4	6.3	3.5	3.5	12	0
6.4	6.3	4.0	4.1	21	219*
6.4	6.3	4.8	4.9	19	483
6.4	6.3	6.9	7.4	37	618
6.4	6.3	7.9	8.0	28	477
6.4	6.3	9.0	8.7	20	0

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TABLE 5

Presporulation pH constant 6.5. Sporulation pH varied with full-strength buffer.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter	Average Count
6.5	6.5	5.2	5.2	22	0
6.5	6.5	5.6	5.6	21	15
6.5	6.5	7.4	7.3	18	25*
6.5	6.5	7.9	7.8	18	0*
6.5	6.5	8.5	8.1	16	0*

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TABLE 6

Presporulation pH constant 6.4. Sporulation pH varied with 1:1, water-buffer solution.

Presporulation pH Initial	Presporulation pH Final	Sporulation pH Initial	Sporulation pH Final	Mycelium Diameter	Average Count
6.4	6.3	4.0	4.1	14	63
6.4	6.3	4.9	5.0	18	49
6.4	6.2	6.2	6.2	21	119
6.4	6.4	7.7	7.8	14	0
6.4	6.4	8.9	8.5	14	0

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*indicates beaded hyphal tips seen

TABLE 7

Presporulation pH varied with buffer. Plain water used for sporulation.

Presporulation pH		Sporulation pH		Mycelium	Average
Initial	Final	Initial	Final	Diameter	Count
4.8	4.8	6.9	6.5	14	65
5.6	5.6	6.9	6.5	20	70
7.1	7.1	6.9	6.5	17	45
7.7	7.7	6.9	6.5	11	13
8.3	8.0	6.9	6.5	7	5

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TABLE 8

Presporulation pH varied with buffer. Sporulation pH varied with 1:1, water-buffer solution.

Presporulation pH		Sporulation pH		Mycelium	Average
Initial	Final	Initial	Final	Diameter	Count
4.8	4.8	5.2	5.1	15	0
5.6	5.6	5.6	5.6	22	0*
7.1	7.1	7.4	7.3	10	0
7.7	7.7	7.9	7.8	6	0
8.3	8.1	8.3	8.1	3	0

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TABLE 9

Presporulation temperature constant 24 C; sporulation temperature varied as indicated.

Sporulation Temperature	Mycelium Diameter	Average Count
8 C	23	87
8	22	86
15	25	203
15	27	220
22.2	23	127
22.2	24	146
24	24	146
24	23	157
36	13	0
36	13	0

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TABLE 10

Presporulation temperature constant 20-21 C. Sporulation temperature varied as indicated.

Sporulation Temperature	Mycelium Diameter	Average Count
0 - 1 C	19	0
3.4 - 5	19	0*
20	25	137
25 - 30	20	0*
34.5	20	0*
36	20	0

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TABLE 11

Presporulation and sporulation temperatures varied

Presporulation Temperature	Hours of Presporulation	Sporulation Temperature	Hours of Sporulation	Diameter of Mycelium	Average Count
0 - 1 C	48	-	-	no growth	
0 - 3	38½	-	-	no growth	
3.5 - 5	24	-	-	1.5 mm growth	
5	38½	-	-	5 branches from germ tube	
15	42½	15 C	24½	20	94
22	39	22.2	25½	19	78
34.1	39	-	-	8 branches from germ tube	

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TABLE 12

Presporulation and sporulation in continuous light.

Mycelium Diameter	Average Count
25	151
27	129
33	107
29	121
26	168
32	124

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TABLE 13

Presporulation and sporulation in continuous darkness.

Mycelium Diameter	Average Count
21	97
22	48
21	51
20	62
20	64
19	85

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TABLE 14

Presporulation: light-darkness; sporulation: darkness-light.

Mycelium Diameter	Average Count
21	101
22	64
19	62
20	91
21	70
21	93

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TABLE 15

Presporulation: darkness-light; sporulation: light-darkness.

Mycelium Diameter	Average Count
20	32
20	49
27	129
20	33

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TABLE 16

Presporulation: light-darkness; sporulation: darkness.

Mycelium Diameter	Average Count
32	99
28	114
35	169
32	139

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TABLE 17

Presporulation: darkness-light; sporulation: darkness.

Mycelium Diameter	Average Count
24	114
25	168
22	112
27	134

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TABLE 18

Presporulation: light-darkness; sporulation: light-darkness.

Mycelium Diameter	Average Count
31	143
30	169
30	120
34	195

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TABLE 19

Presporulation: darkness-light; sporulation: light.[1]

Mycelium Diameter	Average Count
25	166
28	123

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TABLE 20

A comparison of the results of experiments 12 through 19.

Experiment number	Total hours light	Total hours darkness	Avg. Mycel. Diameter	Average Count
12	62	-	29	133
13	-	63 3/4	21	68
14	44	21	21	80
15	21	44	20	61
16	18	49½	32	130
17	24½	43	25	140
18	43	24½	31	157
19	49½	18	27	144

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[1]remainder of cultures in this series were contaminated; no repeat was made.

Fig. 1. Zoosporangia as seen with counting grid in eyepiece of stereoscoptic microscope. (X 27).

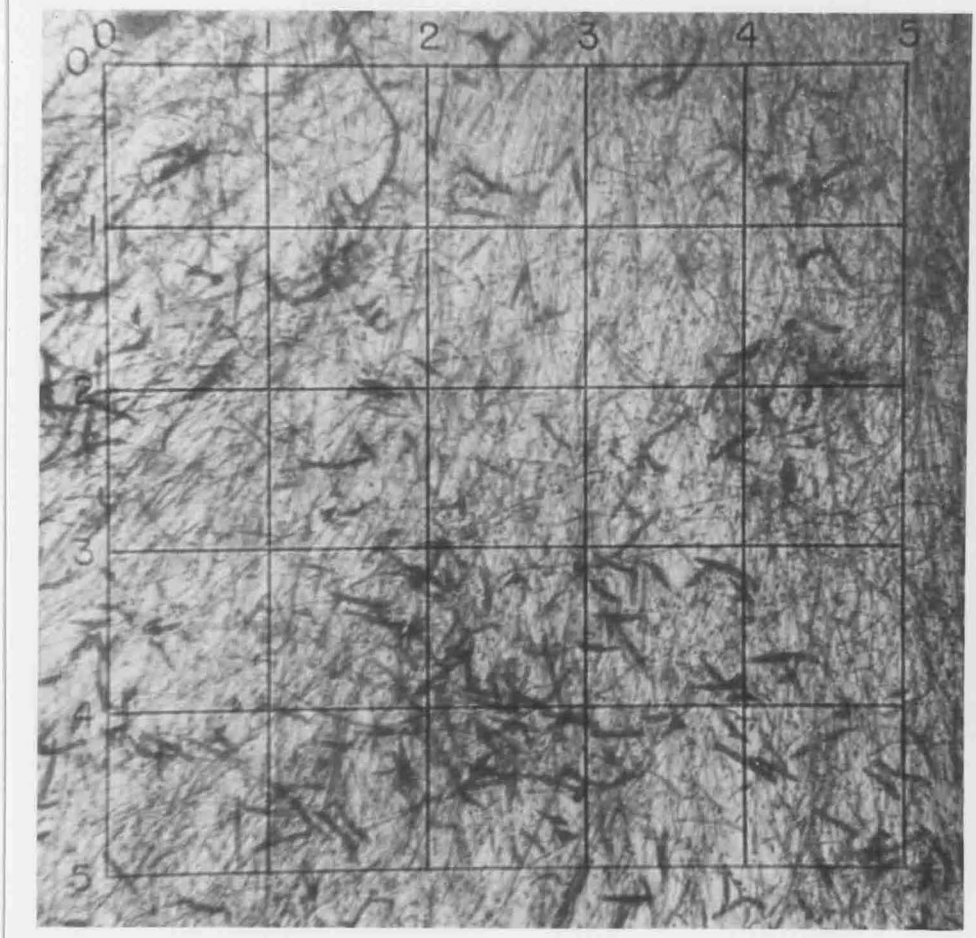

0
1
2
3
4
5

Fig. 2. View of culture showing zonation of zoosporangia. (X 5).

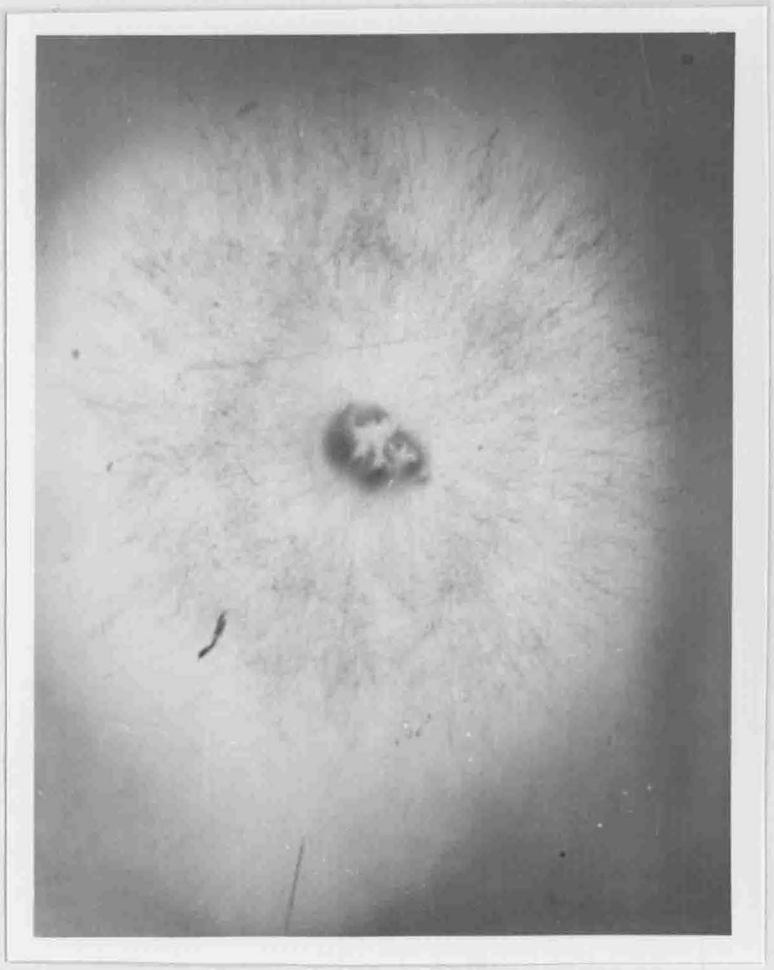


Fig. 3. Beaded hyphal tips found at limiting extremes of pH and temperature. (X 1000).

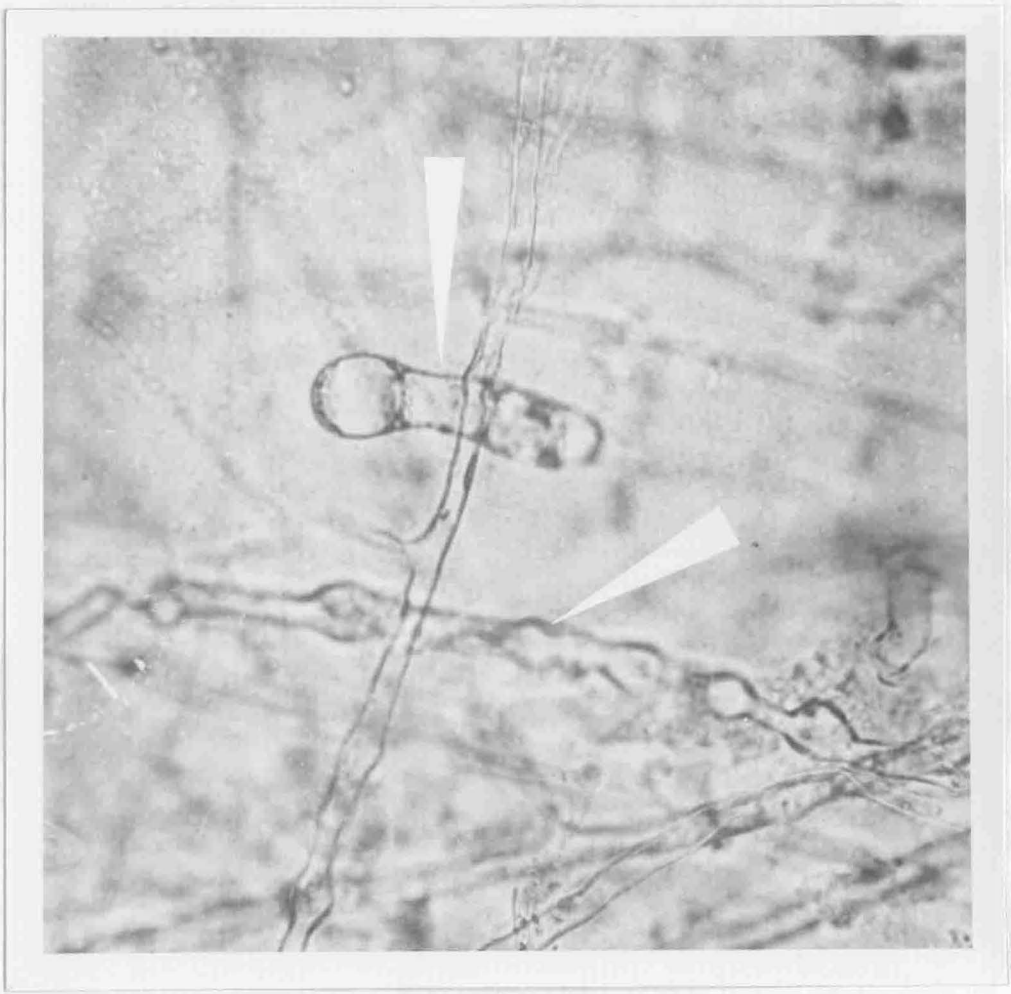
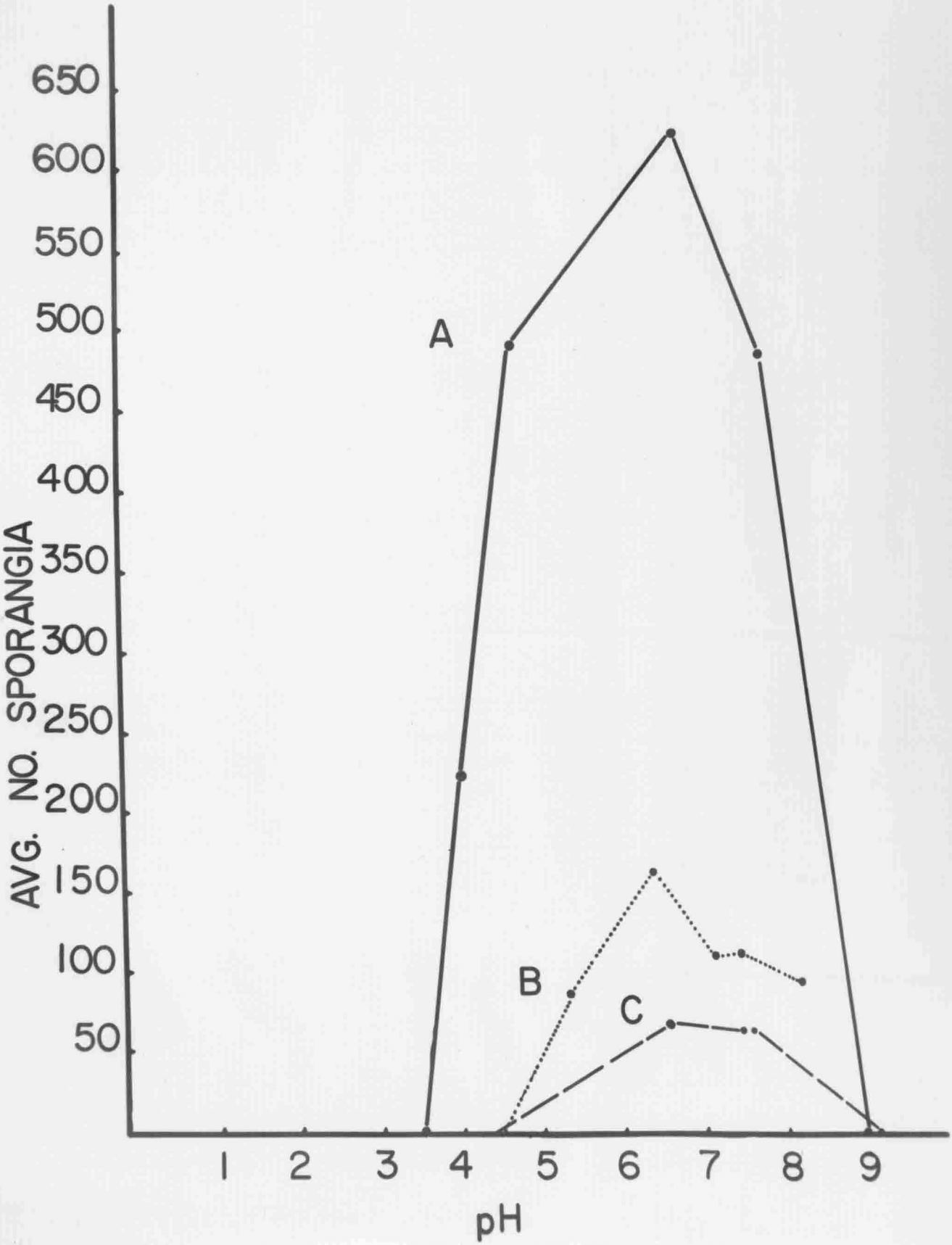


Fig. 4. A comparison of experiments 2, 3 and 4.

A - Exp. 4: Sporulation pH adjusted with buffer; special counting technique.

B - Exp. 2: Presporulation and sporulation pH adjusted with HCl, NaOH.

C - Exp. 3: Presporulation pH not adjusted; sporulation pH adjusted with HCl, NaOH.


650
600
550
500
450
400
350
300
250
200
150
100
50
AVG. NO. SPORANGIA
A
B
C
1
2
3
4
5
6
7
8
9
pH

VITA

Philip Calvin Lee, Jr., was born in Roanoke County, Virginia, on 6 December 1933. He received his primary education in the Botetourt County schools and graduated from Troutville High School in June, 1951. After completing one year at Roanoke College, he served as a member of the U. S. Air Force. He returned to Roanoke College in September 1957, where he majored in Biology and was elected to membership in Beta Beta Beta Honorary Biological Society, and Xi Theta Chi, Honorary Language Fraternity. In August, 1960, he received the B. S. Degree from Roanoke College and was accepted for graduate studies at the University of Richmond. While at the University he was awarded the Williams Fellowship for graduate studies during the 1961-62 session. He received the Master of Arts Degree in Biology from the University of Richmond in August, 1962. He was awarded a Graduate Assistantship at Virginia Polytechnic Institute, where he will continue his graduate studies toward the Doctor of Philosophy Degree.