EFFECTS OF LIGHT AND TEMPERATURE ON THE FORMATION OF SEXUAL STRUCTURES IN THE FAMILY SAPROLEGNIACEAE

by

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Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of

DOCTOR OF PHILOSOPHY

in

Botany

September, 1965

Blacksburg, Virginia

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INTRODUCTION

Few attempts have been made to control conditions of illumination during artificial culture of the saprolegniaceous fungi. Research studies should be based on rigid control and standardization of environmental conditions. In several instances, light has been shown to induce the formation of sexual structures in fungi (Leach, 1963). In most instances, wave lengths in the ultraviolet and blue regions of the spectrum were found to be most effective in inducing sporulation. Stevens (1929) reported that ultraviolet irradiation produced abundant sexual spores in Glomerella cingulata, which fruited poorly in ordinary laboratory culture. My previous work with Saprolegnia parasitica Coker (Lee, 1962) showed that at temperatures lower than optimum for sporulation, the presence of light resulted in an increase in the number of zoosporangia formed. These works indicated that light might affect the sexual sporulation of the saprolegniaceous fungi and that there might be an interrelationship between the effects of light and temperature.

Cochrane (1958) emphasized that any simple explanation of the effects of light was made more difficult because of the two extreme types of response during exposures of fungi

to alternate periods of light and darkness. One type, such as Sclerotinia fruticola sporulated well in darkness, less or not at all during the light period. Other fungi such as Fusarium spp. sporulated poorly in darkness but formed zones of spores after even brief exposure to light.

The object of the present research was to determine if combined light and temperature variations exerted any effect on the growth and sexual sporulation of selected saprolegniaceous fungi, and to show any such differences on a quantitative basis of oogonium counts, mycelium measurements and dry weights. If such differences were found, this would indicate the importance of the proper control of illumination during artificial culture of these fungi, and further indicate the illumination conditions that would be most favorable to the formation of the sexual structures required for species identification.

LITERATURE REVIEW

The literature concerning the effects of light and temperature on various fungi is extensive. Marsh (1959) compiled a guide to the literature concerning the effects of light on reproduction, morphology, pigmentation and phototrophic responses in fungi.

It has been found that in general, visible light may exert any of a number of influences on the morphology and physiology of fungi. Cochrane (1958) divided the fungi into two rather broad groups according to the effects of light on sporulation. In one group, the fungi sporulated well in darkness but poorly or not at all in light and in the other group the fungi sporulated well in light but poorly or not at all in darkness. Ramsey and Bailey (1930) demonstrated that profuse sexual sporulation took place in species of Macrosporium and Fusarium when these sparsely fruiting cultures were exposed to ultraviolet light. Barnett and Lilly (1950) found that Choanephora cucurbitarum failed to form conidia in continuous light or total darkness, but in alternating periods of light and darkness, numerous conidia were produced. Cantino and Horenstein (1956) found that Blastocadiella emersonii

grew more rapidly in light than in darkness and that light induced an increase in carbon dioxide fixation. Fergus and Schein (1963) found that in Physarum gyrosum, light intensity of 440 ft-c caused rapid fruiting and that no fruiting took place in darkness. In work with Phytophthora, Harnish (1965) found occasional oospores formed in continuous light of 700 ft-c intensity and abundant oospores were formed in reduced light intensity or darkness.

The literature concerning the saprolegniaceous fungi reflects the same varied responses to illumination. Working with Saprolegnia mixta de Bary, Klebs (1899) found no noticeable differences in mycelial growth or zoospore formation among cultures grown in diffuse daylight and those grown in darkness. In his monograph of the Saprolegniaceae, Coker (1923) grew the fungi on various natural substrates but did not control light and temperature conditions. Duff (1929) worked with Saprolegnia sp. and reported the optimum growth temperature was 20 to 25 C, and that growth was not affected by sunlight coming through ordinary glass. Cotner (1930) surveyed a number of saprolegniaceous fungi and listed the temperature optima for asexual sporulation. No mention was made of

illumination control. Kanouse (1932) was the first person to observe sexual structures in Saprolegnia parasitica Coker in artificial culture. Again, no mention was made of attempts to control lighting conditions. Varitchak (1934) studied sexual reproduction in Saprolegnia sp. and found that oogonia and antheridia were formed after 10 to 14 days when the cultures were kept at 8 C to 10 C on carrot extract with two per cent agar. Development of sexual structures was retarded when the medium was more than 2 mm thick. Temperatures of less than 6 C appeared to favor formation of sexual organs. No mention was made of illumination control. Blank and Tiffney (1936) reported that peptone agar exposed to ultraviolet light was effective in ridding Saprolegnia sp. cultures of bacterial contamination. Evidently some product was formed in the agar that was more injurious to the bacteria than to the fungus, or this might have indicated that the fungus was more resistant than the bacteria to the products formed. Ziegler (1948) found that Thraustotheca primoachlya required light for zygote germination when cultures were placed on a laboratory table at approximately equal temperatures in darkness and diffuse daylight. Zygospores of Protoachlya hypogyna and Achlya recurva kept in darkness for 72 hours

did not germinate. When these cultures were placed in daylight for an additional 72 hours, germination took place. Etzhold (1960) found that oogonial formation in Saprolegnia ferax (Gruith) Thuret was inhibited by light coming from filters and cellophane transmitting mainly red light. Lee (1962) demonstrated that light from fluorescent tubes stimulated growth and asexual sporulation in S. parasitica. At temperatures lower than optimum for sporulation, the presence of light resulted in an increase in the number of sporangia formed. In cultures exposed to intermittent periods of light and darkness, those that received the longest duration of light produced the greatest number of zoosporangia, even at lower than optimum sporulation temperature.

It has been reported that the effects of light on fungal growth and reproduction are more difficult to demonstrate on artificial media than on natural media. The works cited in this review used either natural or chemically undefined synthetic media. Too few workers have investigated the possibility of changes in the medium brought about by light, which in turn might effect the growth and sporulation of the fungus. Control conditions were poor in many instances and

temperature was not controlled in several studies. Most of the studies cited were of a qualitative rather than quantitative nature.

The present investigation was based on the use of a chemically defined medium, carefully controlled cultural conditions and results tabulated on the basis of oogonium counts, mycelium measurements and dry weights.

MATERIALS AND METHODS

Stock Cultures:

The fungi used in the experiments came from the culture collection of Dr. W. W. Scott, Virginia Polytechnic Institute. The origins of the cultures were:

Achlya americana Humphrey; Saprolegnia ferax (Gruith) Thuret: Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Saprolegnia parasitica Coker (isolate 2-27-59); S. parasitica Coker (isolate 6-28-60): Isolated from diseased fish (Scott and O'Bier, 1962).

Stock cultures were maintained at 15 C in darkness on agar plates containing the same medium concentration as the standard medium described below. Stock cultures were kept bacterial-free by the glass ring technique (Raper, 1937). The stocks were sub-cultured by hyphal tip inoculation after periods of ten days.

Media:

Water used in the experiments was distilled by a steam still and run through a Barnstead Bantam Model I demineralizer column containing research grade REXYN I-300 demineralizer. One of the first major obstacles

that had to be overcome in this work was that of obtaining a suitable medium. The medium suggested by Scott, Powell and Seymour (1963) allowed very rapid growth of Saprolegnia and a much slower growth rate of Achlya. Sexual sporulation took place in both genera in the medium after about two or three weeks. A medium was required that would give good vegetative growth, fairly rapid sporulation and approximately equal times required for sporulation in all isolates. Since the saprolegniaceous fungi usually form sexual structures when most of the nutrient supply of the medium has been depleted, a standard medium was devised by trying several dilutions of the full strength medium. Attempts were also made to vary the carbon to nitrogen ratio (Barksdale, 1962) in order to increase sexual sporulation. The dilution method was found to be more successful than changes in the carbon to nitrogen ratios. One part full strength medium to one part of ion free water was too dilute and caused the formation of zoosporangia in the cultures. Several other dilutions were attempted using all the isolates as test organisms. The dilution of three parts full strength medium to two parts ion free water was found to be in the desired range and was selected for the standard medium.

This dilution gave comparable growth and sporulation rates in all the test isolates that fruited. The medium constituents are shown in Table 1. For convenience, a full strength medium was prepared and a three parts medium to two parts water dilution was used as the standard medium throughout the experiments. In preparing the full strength medium, ethylenediaminetetraacetic acid was dissolved in 500 ml boiling water. The other constituents were added in the order shown in Table 1. Water was added to bring the level to one liter. Nine hundred milliliters of this full strength medium were diluted with water to 1500 m. The pH of the medium was adjusted with KOH to 6.7 using a Fisher Accumet pH meter. The medium was heated again and 26 ml aliquots were pipetted into 15 cm plastic screw-cap Pyrex test tubes. Each tube delivered 25 ml to a petri dish. The medium was autoclaved at 15 pounds pressure for 15 minutes. The medium was stored in light-tight boxes until used. Standard, nine centimeter Pyrex petri dishes were used in all experiments. All glassware was washed in hot 7X detergent solution, rinsed twice with tap water and once with distilled water before sterilization in a hot air oven.

TABLE 1. Constituents of media.

Constituent and Source		Grams per Liter	
		Full Strength	Standard
KEDTA	Fisher Certified Reagent	0.5000	0.3000
K_2HPO_4	"	0.1833	0.1099
KH_2PO_4	"	0.1167	0.0700
$MgCl_2 \cdot 6\ H_2O$	"	1.2000	0.7200
$CaCl_2 \cdot 2\ H_2O$	"	0.0358	0.0215
$MnCl_2 \cdot 4\ H_2O$	"	0.1430	0.0858
$ZnCl_2$ (anhydrous)	"	0.0834	0.0501
$FeCl_3 \cdot 6\ H_2O$	"	0.0050	0.0030
Sodium Glutamate	"	2.0000	1.2000
DL Methionine	"	0.0500	0.0300
Glucose	Baker's Analyzed Reagent	5.0000	3.0000
Purified Agar	DIFCO	-	1.5000
KOH	Fisher Certified Reagent	-	to pH 6.7
Ion-free Water		Dilute to one liter	

Culture Methods:

Single zoospores were used for inoculum. A small block of agar containing mycelium was cut from a stock culture plate and the block was placed in sterile water to induce zoospore formation. The petri dish was incubated in darkness for 24 hours after which time one milliliter of zoospore containing water was pipetted aseptically onto the surface of an agar plate. The agar plates were prepared by adding 1.5% purified agar to the standard medium. After twelve hours incubation in darkness at 15 C, the germinated zoospores were removed singly with the aid of a dissecting microscope, and used to inoculate plates of medium. A flattened piece of Chromel-A wire was used to cut out a very small cube of agar surrounding the germinated zoospore. After inoculation the cultures were incubated under experimental light and temperature conditions. Controls for each experiment were incubated in darkness. Five replicates, the maximum number permitted because of space limitations, were used except in cases noted. At the end of the incubation period the cultures were killed by pouring about 10 ml of 70% ethyl alcohol into each plate. Alcohol was found to be the least noxious killing agent, did not distort

the oogonia or cloud the medium and caused very little shrinkage of the mycelium during the oogonium counts.

Measurements:

After killing the cultures, the spent medium and alcohol were drained from the petri dish and the mycelium was spread evenly over the bottom of the dish. The dish was placed on the stage of a binocular dissecting microscope and the mycelium diameter was measured with a metric rule. The averages of two measurements are shown in Tables 2, 3, 4, 6 and 7.

Oogonium Counts:

The counting method used was a modification of that used by Pieters (1915). All counts were made at a magnification of 45 X, using a binocular dissecting microscope. A Whipple disc counting grid was placed in the eyepiece of the microscope and the disc was calibrated with a stage micrometer. The entire field of view within each area counted was 5.43 square mm, which was divided into 100 smaller squares by the grid (Fig. 1). The petri dish containing the drained mycelium was placed on the microscope stage so that the grid covered the edge of the mycelium. Oogonia in

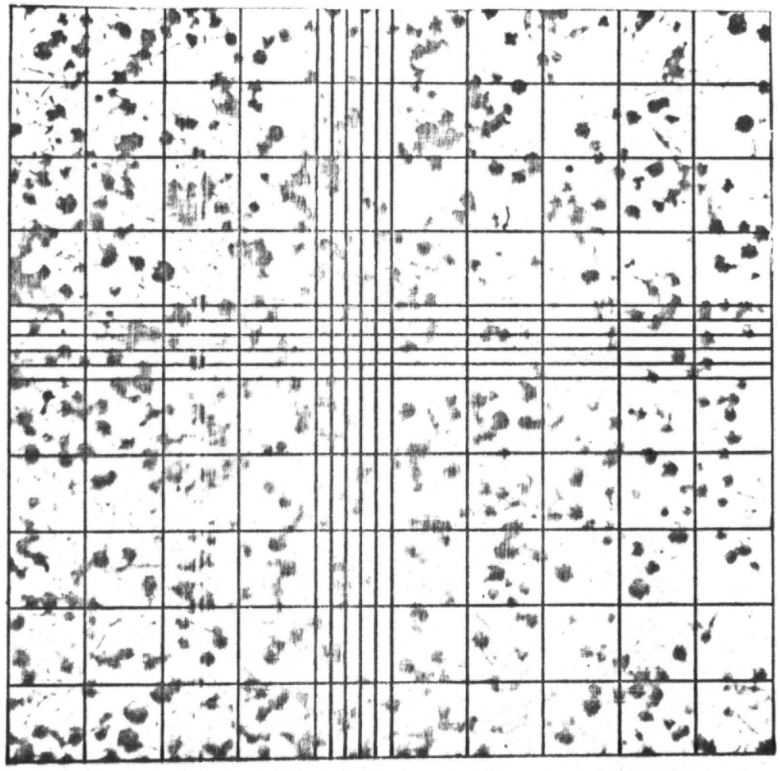


FIG. 1. Typical view of oogonia through counting grid.

that area were counted. The dish was moved so that the next adjacent area could be counted. All oogonia in a linear series of adjacent areas across the diameter were thus counted. The number of areas counted was determined by the colony diameter. Immature and mature oogonia were counted together. Counts per area were recorded with a hand tally counter. The grand total of oogonia counted per culture was divided by the number of areas on that culture. This gave a relative number that could be compared with other cultures under varied experimental conditions.

Dry Weight Determinations:

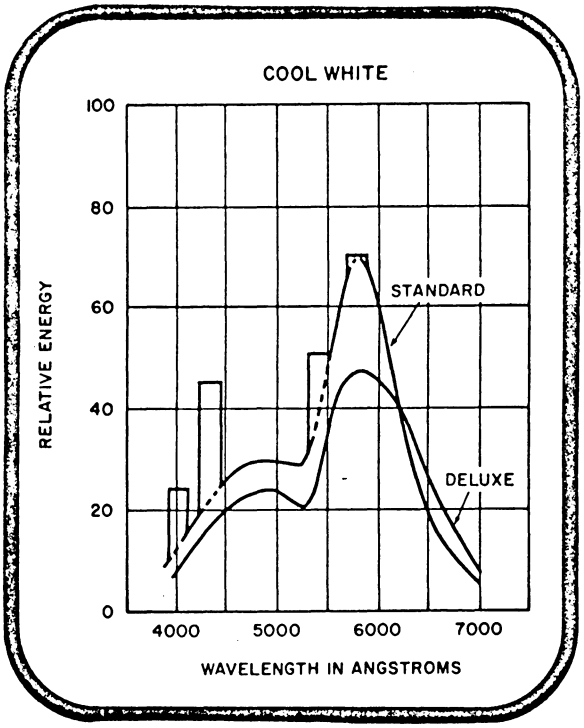
After the oogonia were counted, each culture was suction filtered onto a Whatman No. 1 filter paper circle that had been dried at 90 C for 24 hours and weighed. During filtration the mycelium was rinsed twice with hot water to remove any adsorbed medium. After filtration the filter paper containing the mycelium was dried for 24 hours at 90 C. The gain from the original weight of the filter paper was taken as the mycelium dry weight.

Growth Chambers:

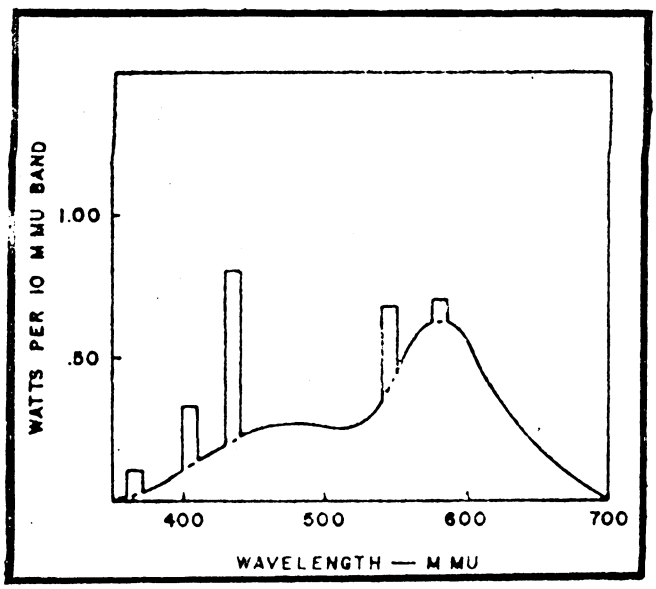
A walk-in constant-temperature room, a Percival Model E57 Environator and two laboratory refrigerators were used as growth chambers. The temperatures were checked originally with a revolving thermograph. Taylor maximum-minimum registering thermometers and laboratory thermometers immersed in water were used inside the chambers to check any temperature variations. Air temperatures did not vary more than plus or minus one degree centigrade and the water immersed thermometers showed relatively constant temperatures.

Light Sources:

Two 20 watt Ken-Rad cool white fluorescent tubes were suspended over the culture dishes and a variable transformer was used to control the light intensity. These tubes were the white light illumination source. The spectral energy distribution curve for these tubes is shown in Fig. 2. A Weston Model 614 foot candle meter was used to determine light intensity. Light intensity was measured in the area where the cultures were to be placed. Readings were made through a clean petri dish cover since the Pyrex glass decreased the intensity about 5 ft-c. Readings were checked at the end of each experiment. A 15 watt General Electric germicidal lamp (2537 Å



a.



b.

Fig. 2. Spectral energy curves: a., Ken-Rad fluorescent tubes (courtesy Westinghouse Electric Corp.); b., Sylvania fluorescent tubes (courtesy Sylvania Electric Corp.).

peak) was used for ultraviolet studies. The lamp was allowed to warm up for 15 minutes prior to exposing the cultures. The petri dish covers were removed and the cultures were irradiated 28 cm from the lamp for an exposure of two minutes unless otherwise noted. Irradiation was done at night and the laboratory was kept in darkness during exposures. After exposure the cultures were placed in light-tight containers except photoreactviation studies.

Filtered Light:

The Percival Environator was used for filtered light studies. Banks of 40 watt Sylvania cool white fluorescent tubes were individually switched to control light intensity. Spectral data for these lamps are shown in Fig. 2. DuPont red and blue cellophane were used to make filters. The fiberglass heat shield between the light banks and the chamber proper was removed and covered with four layers of red or blue cellophane. More layers of cellophane would have given more red or blue light but would have cut the light intensity below 100 ft-c, the lower limits of white light experiments. These filters did not give monochromatic light but reduced the passage of other colors. A Bausch and Lomb spectroscope was aimed at

the filtered light source inside the chamber and the visible wave lengths transmitted to the area of the cultures were: Blue cellophane, 407-710 millimicrons; Red cellophane, 600-700 millimicrons.

RESULTS

Saprolegnia ferax:

Fig. 3 shows a comparison of oogonium formation in cultures grown in darkness, white light (250 ft-c), white light (100 ft-c), red light (100 ft-c) and blue light (100 ft-c) at 15 C. Darkness controls formed the greatest number of oogonia and the rate of formation was highest. Cultures grown in red light gave the next highest rate and absolute number followed by cultures grown in 250 ft-c white light. The lowest rate and absolute numbers were in cultures incubated in 100 ft-c of white or blue light.

Table 2 lists a comparison of physical measurements of the same cultures. The measurements show that cultures incubated in white light had a lower growth rate to begin with but after twelve days incubation these cultures were comparable to the other cultures in dry weight and diameter. After seven days incubation in red light, cultures showed a higher dry weight but repeats of these experiments showed the red light series to be very close to white light series with regard to dry weight and mycelium diameter. Exposure of inoculated plates to two minutes ultraviolet light decreased the rate of growth and sporulation when

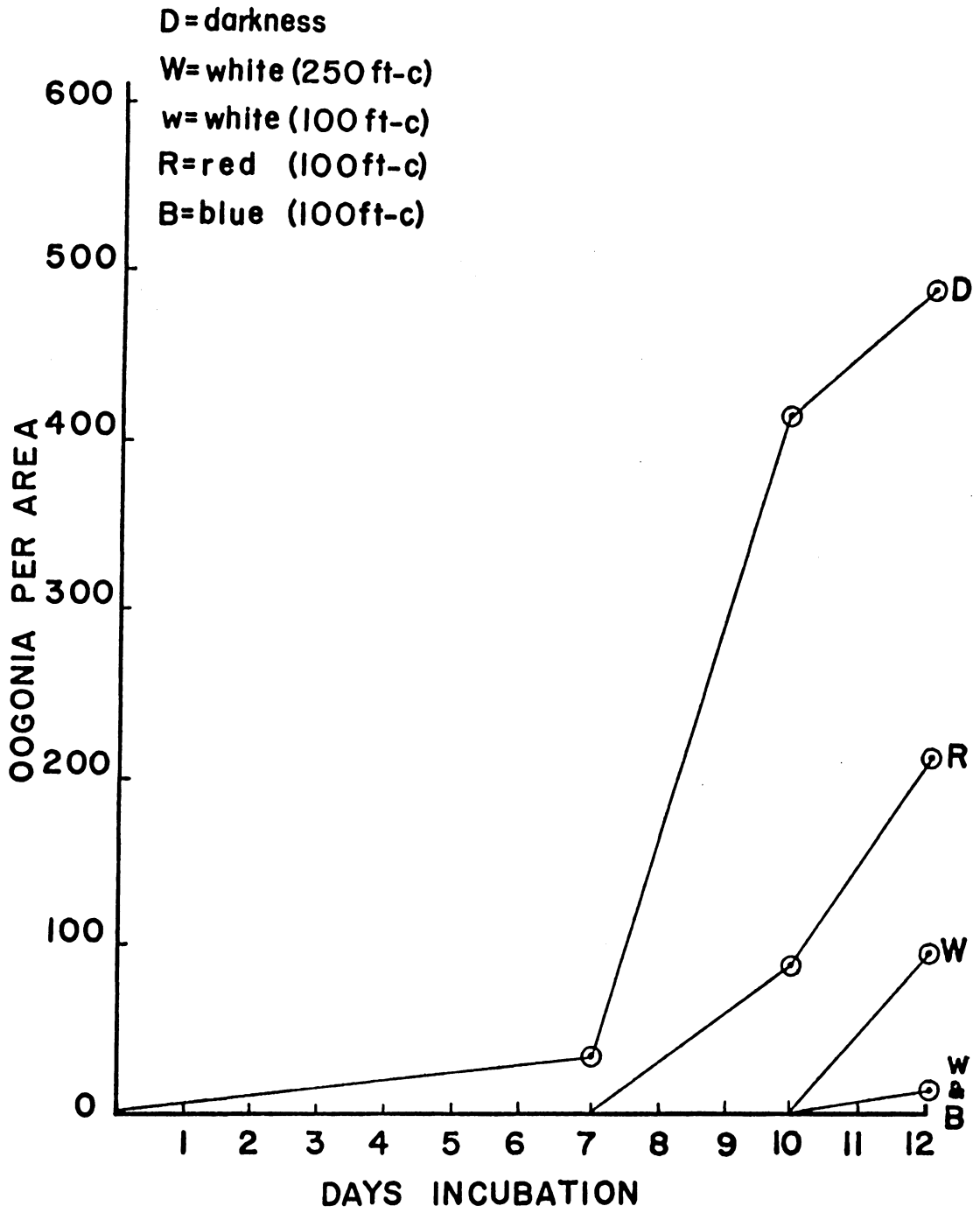


Fig. 3. Oogonium formation in S. ferax at 15 C.

TABLE 2. Comparison of physical measurements of S. ferax cultures at 15 C.

Illumination Conditions	Days of Incubation					
	7		10		12	
	Dia. mm	Wt. mg	Dia. mm	Wt. mg	Dia. mm	Wt. mg
Darkness	84	13	91	24	89	16
White Light (250 ft-c)	77	12	87	23	88	16
White Light (100 ft-c)	-	-	-	-	90	17
Red Light	77	21	90	17	92	18
Blue Light	-	-	-	-	90	19

incubation in darkness followed irradiation. If the irradiated cultures were incubated in light, the inhibition of growth and oogonium formation was considerably lessened (Fig. 4). After seven days incubation at 15 C the control replicates averaged 84 mm in diameter with 32 oogonia per area. Cultures incubated in 250 ft-c white light averaged 73 mm in diameter with nine oogonia per area. Those exposed to two minutes ultraviolet light followed by incubation in darkness averaged 64 mm in diameter with no oogonia. Approximately 94 % photoreactivation was shown with regard to mycelium diameters.

At 25 C no oogonia were formed in either light or darkness. Gemmae were formed profusely in these cultures after six days. Vegetative growth was stimulated under 250 ft-c white light at 25 C. Table 2 shows that at 15 C darkness controls and light series replicates averaged approximately 90 mm in diameter with a dry weight of 24 mg after ten days incubation. At 25 C cultures incubated in 250 ft-c light had reached 90 mm diameter by the seventh day and averaged 40 mg dry weight. Darkness controls at 25 C were 65 mm in diameter with a dry weight of 15 mg (Fig. 5-6). It was interesting to note that cultures grown on a simple medium consisting of

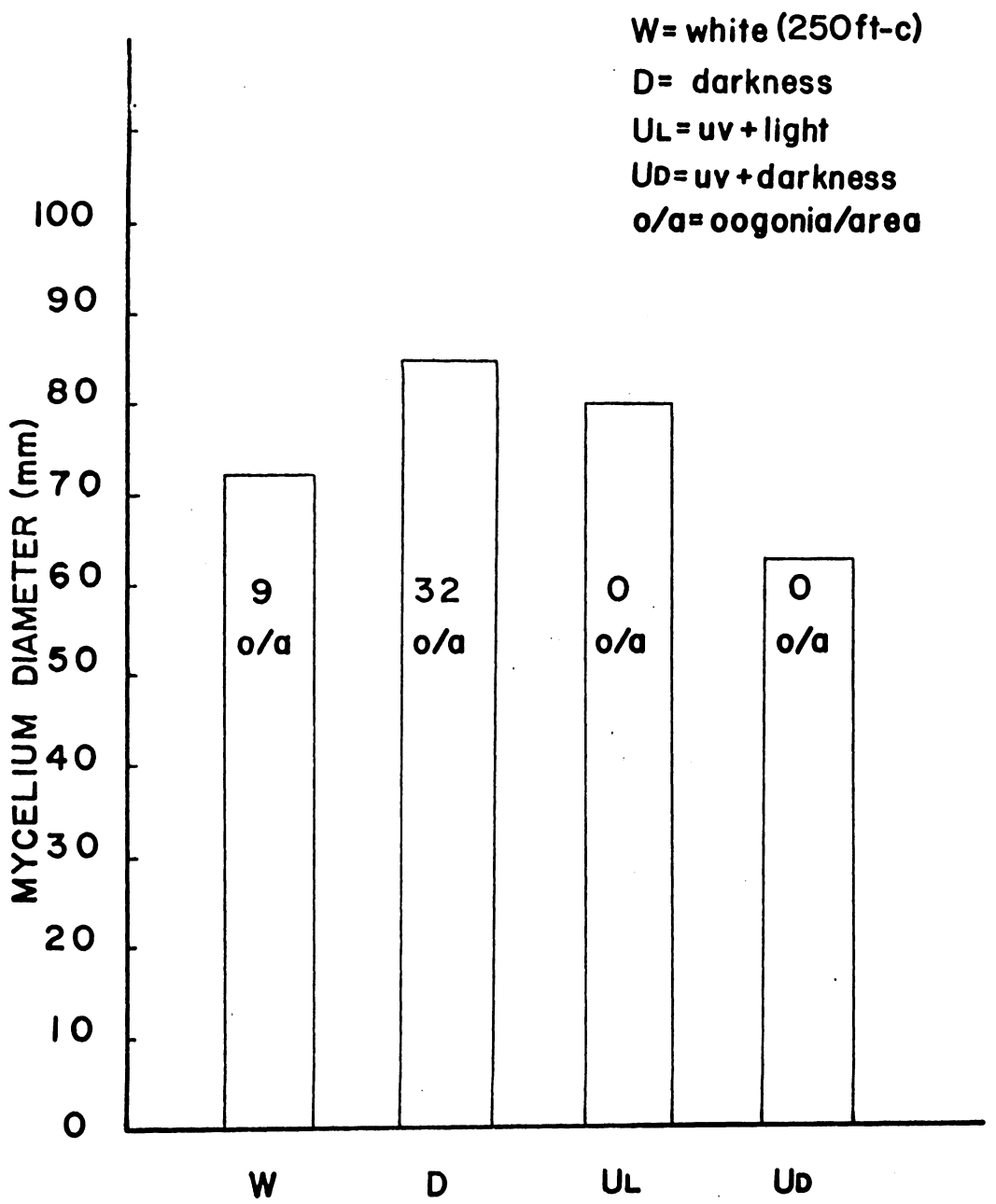


Fig. 4. Mycelium diameters and number of oogonia per area in S. ferax cultures after 7 days incubation at 15 C.

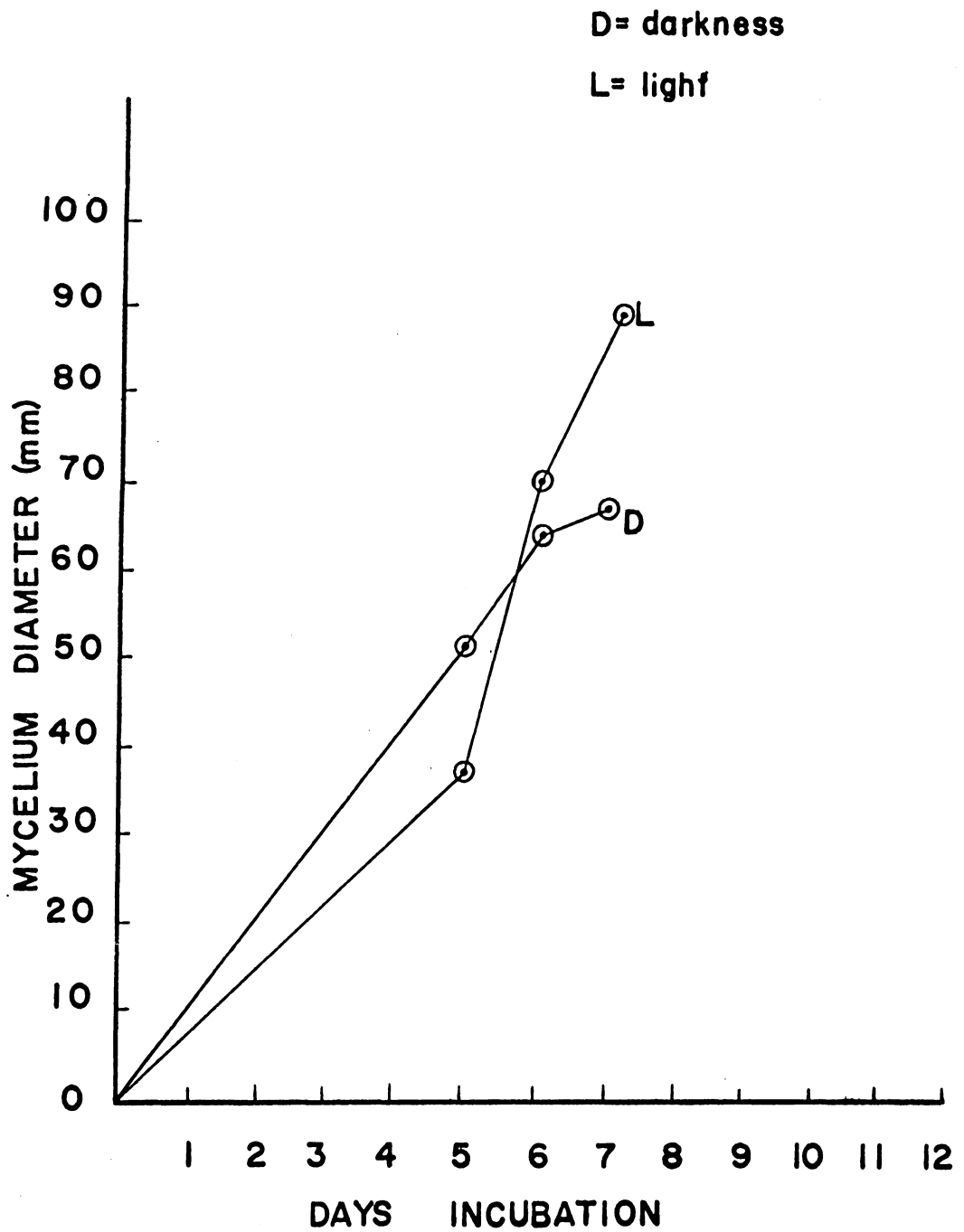


Fig. 5. S. ferax grown in standard medium at 25 C.

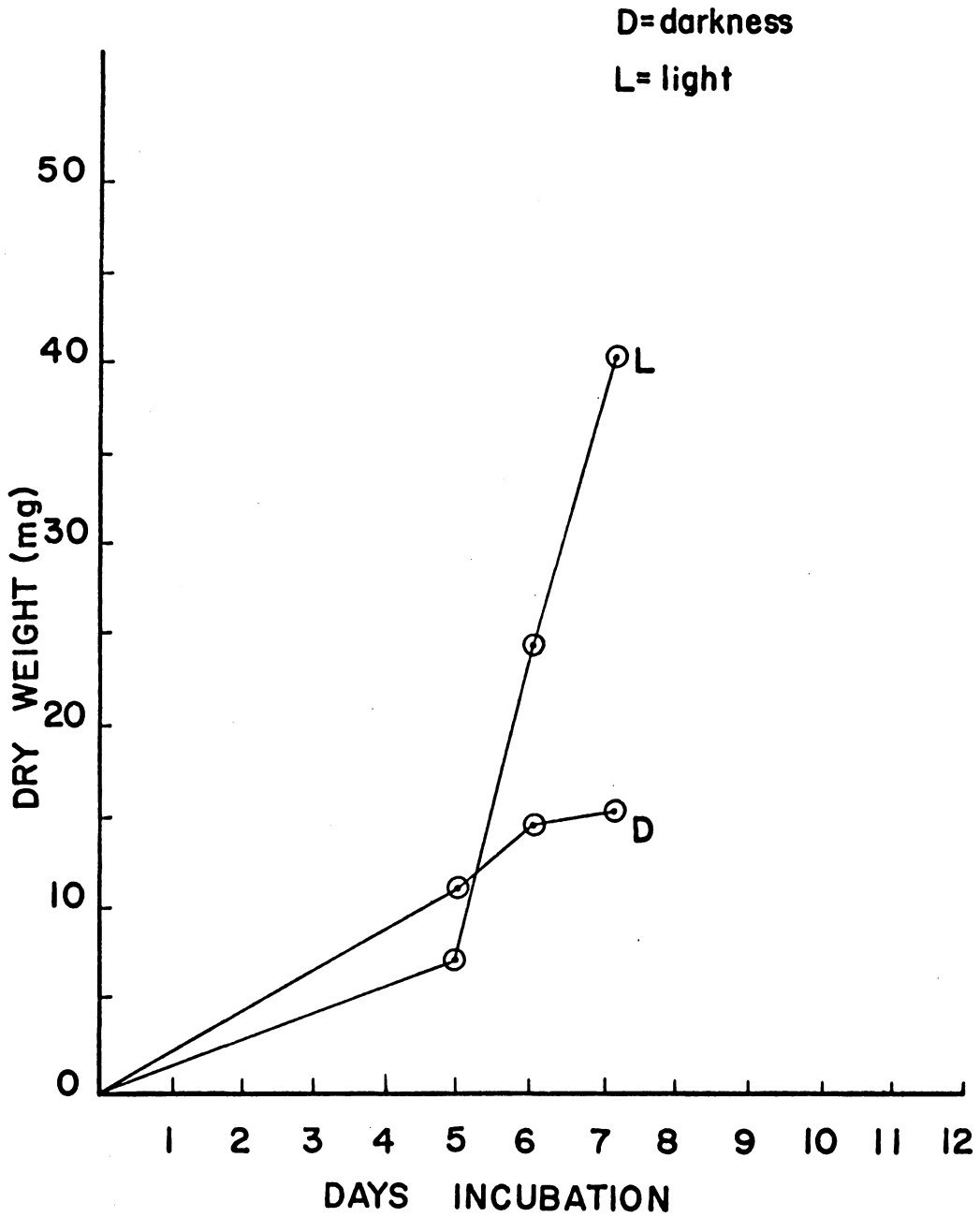


Fig. 6. S. ferax grown in standard medium at 25 C.

glucose (5 g/l), soluble starch (5 g/l) and yeast extract (1 g/l) and incubated at 15 C closely parallelled the growth pattern of cultures grown on the standard medium at 25 C (Fig. 7-8).

Achlya americana:

Preliminary experiments with the full strength medium are shown in Fig. 9. Fourteen days incubation at 15 C was required for oogonium formation as compared with eight days incubation using the standard medium. Cultures grown in darkness were larger in diameter and dry weight than those grown in light (250 ft-c). At 25 C the darkness controls were larger in diameter and dry weight than cultures incubated in 250 ft-c light using the standard medium (Fig. 10). Two minutes ultraviolet exposure inhibited both growth and oogonium formation rates at 25 C. Further experiments showed that 25 C was near the optimum for growth and oogonium formation. Tables 3 and 4 show comparisons of the physical measurements of cultures incubated at 15 C and 25 C in the standard medium. Fig. 11 shows a comparison of oogonia per area formed under 250 ft-c white light and darkness at both 15 C and 25 C using the standard medium. Controls at 15 C began to form oogonia after seven days; cultures

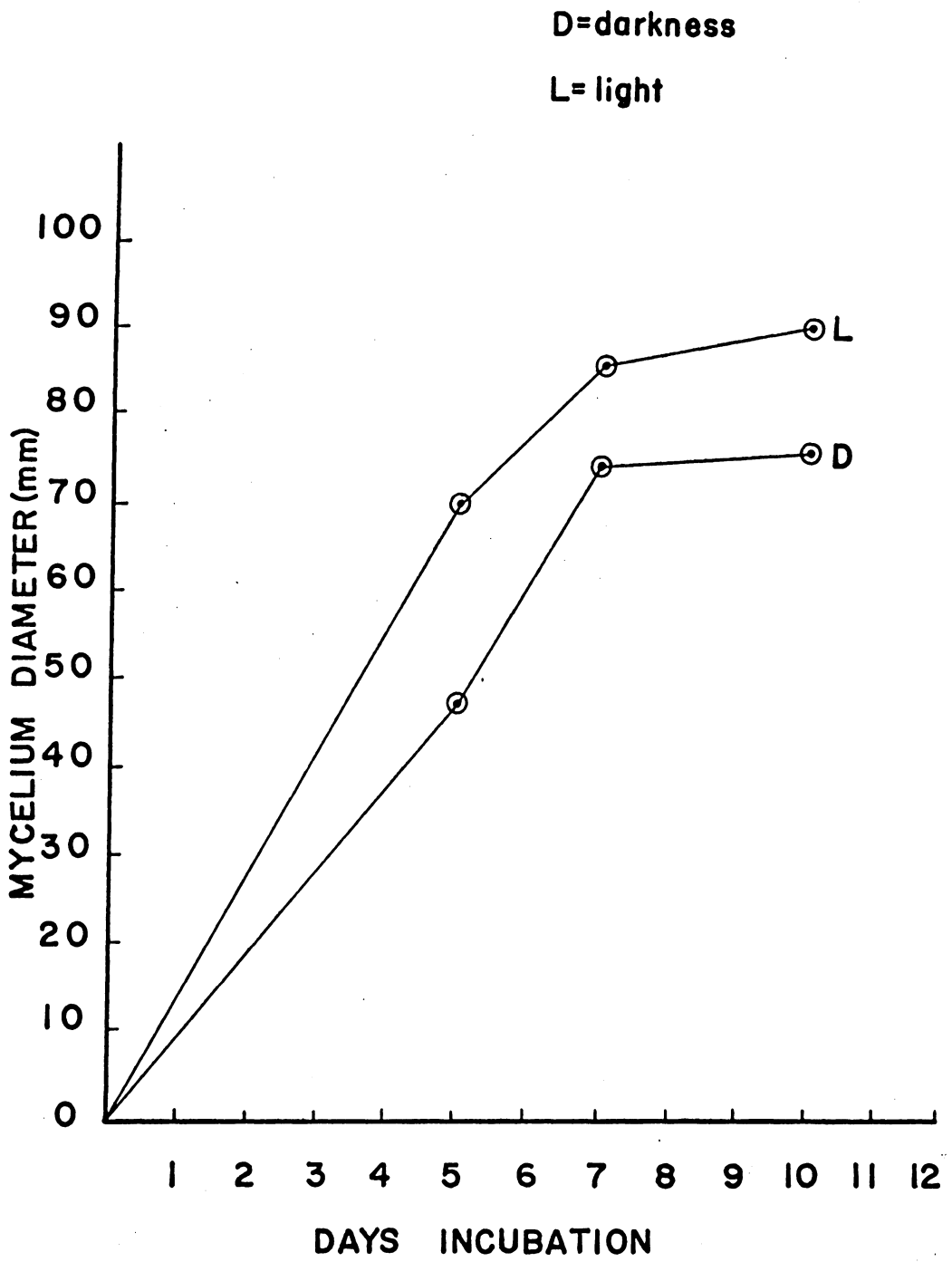


Fig. 7. S. ferax grown on simple medium at 15 C.

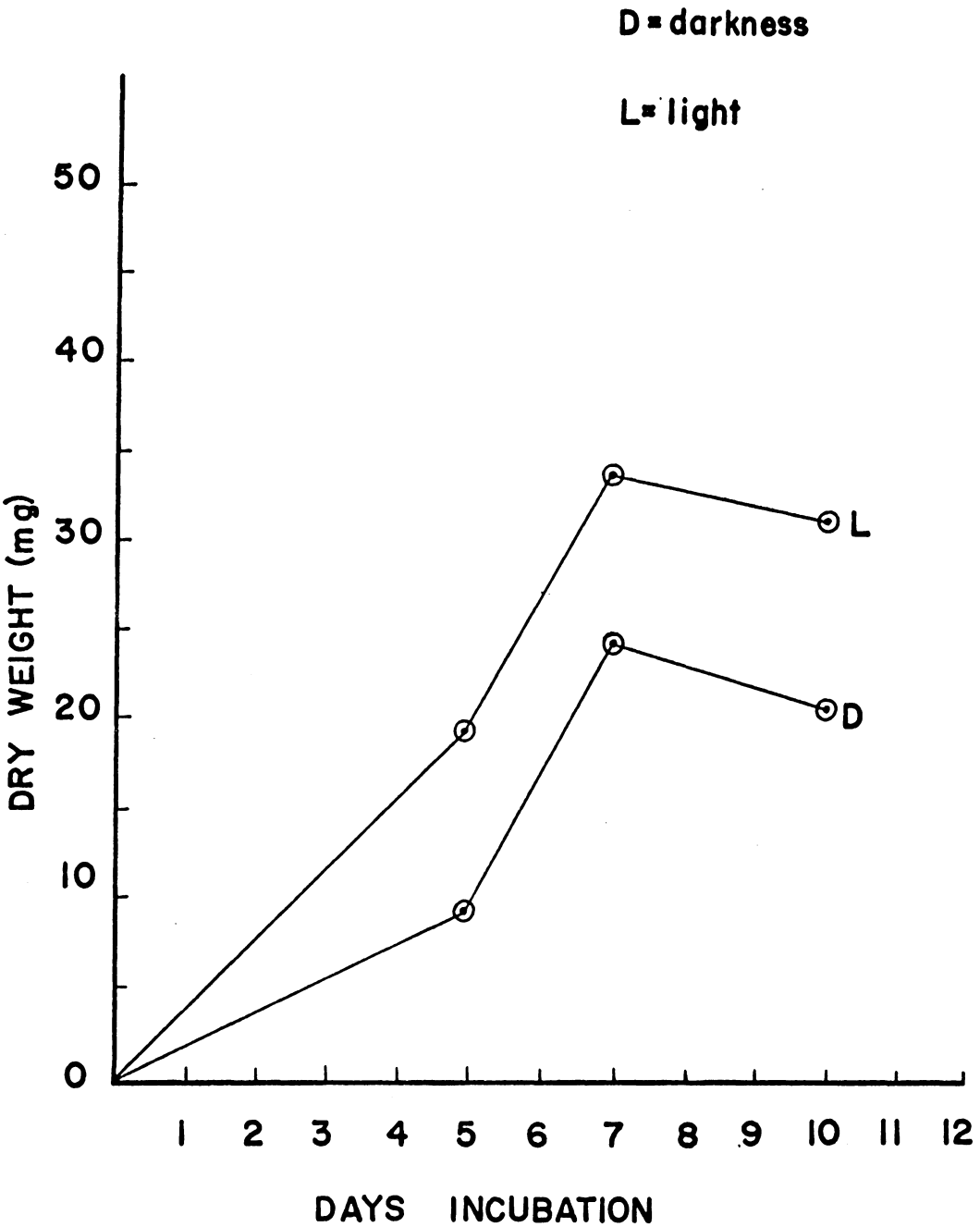


Fig. 8. S. ferax grown on simple medium at 15 C.

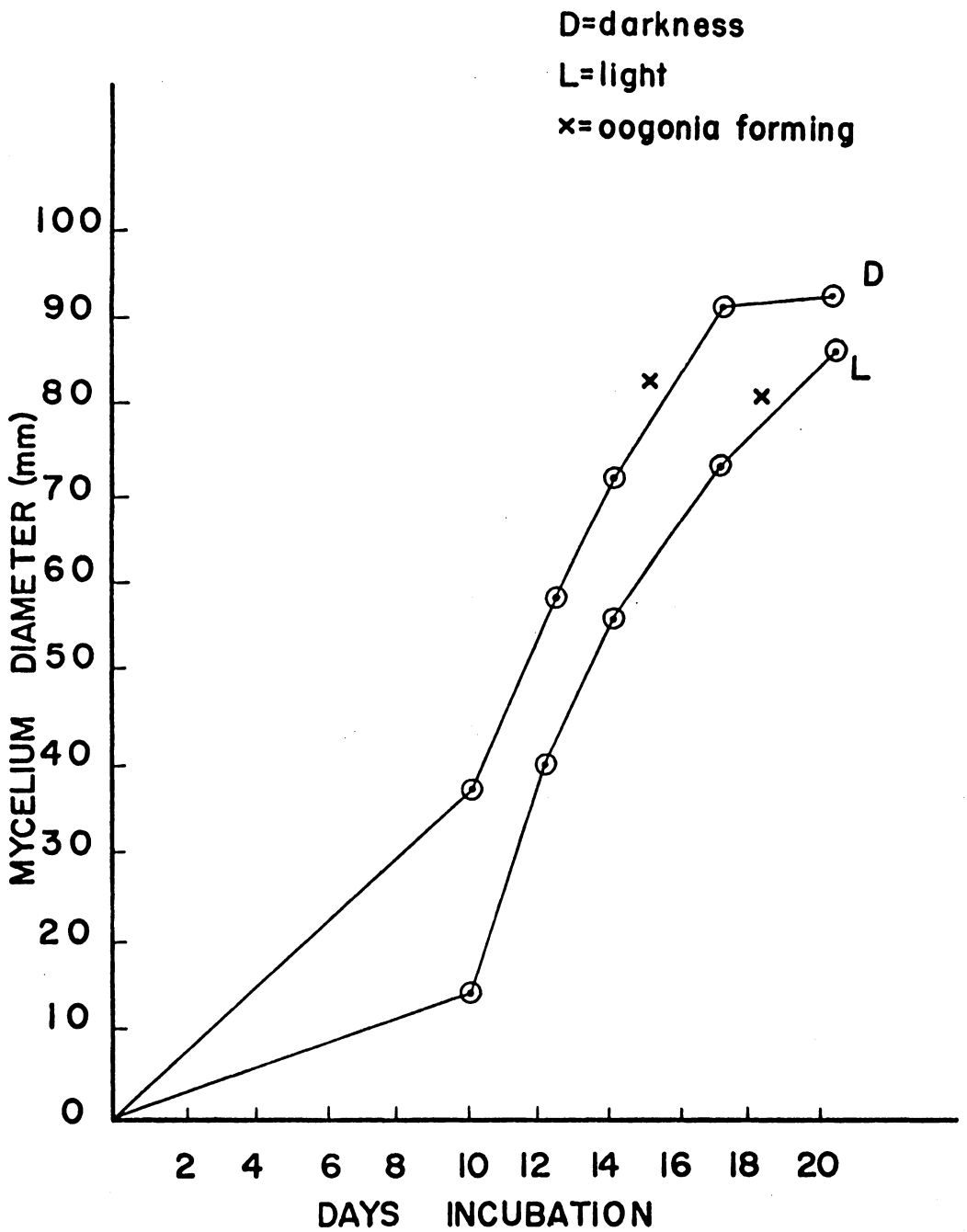


Fig. 9. A. americana grown on full strength medium at 15 C.

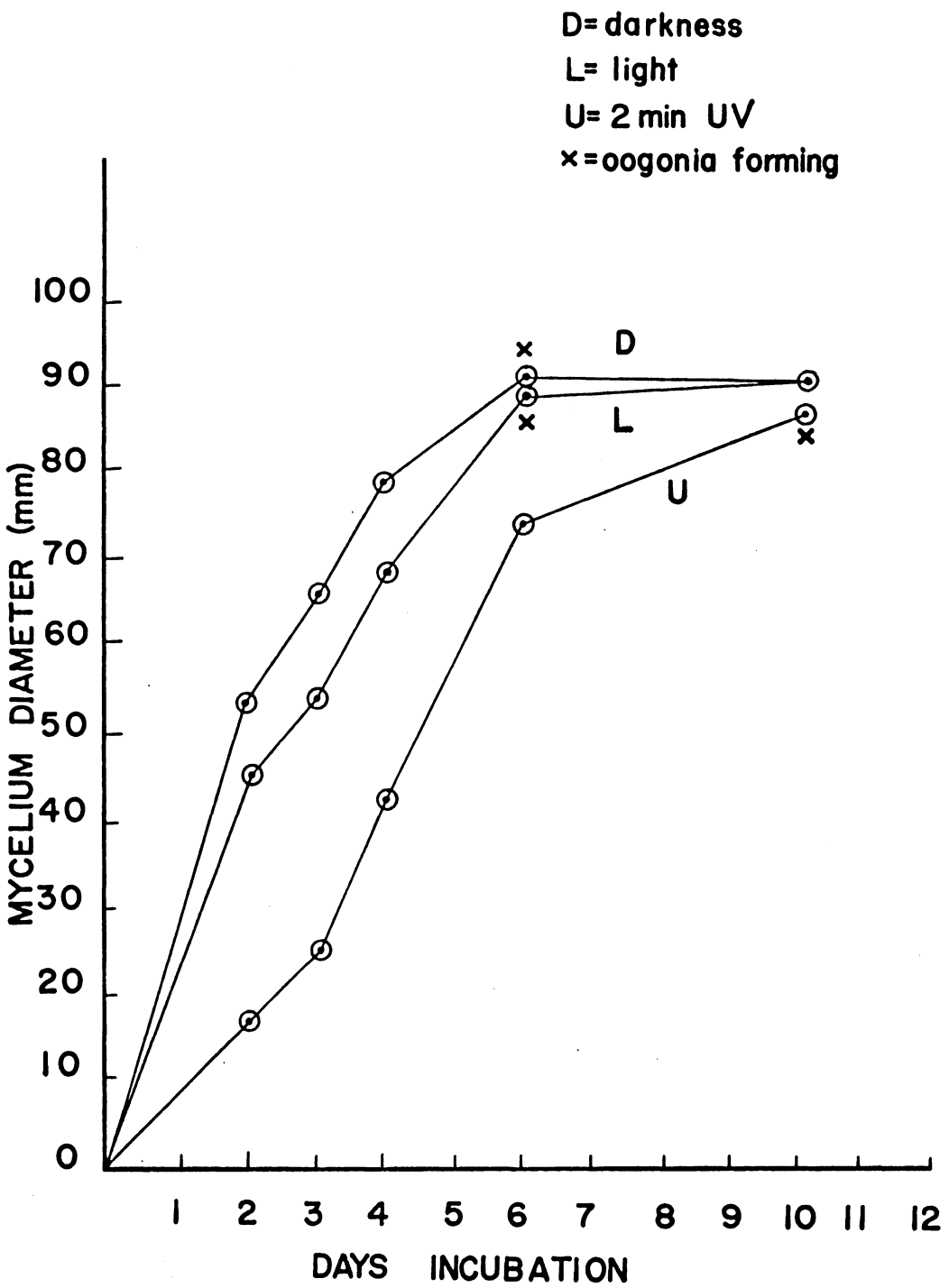


Fig. 10. A. americana grown in standard medium at 25 C.

TABLE 3. Physical measurements of A. americana cultures grown at 15 C in standard medium.

Illuminating Conditions	Days of Incubation							
	7		8		10		12	
	Dia. mm	Wt. mg	Dia. mm	Wt. mg	Dia. mm	Wt. mg	Dia. mm	Wt. mg
Darkness	24	2.6	28	1.9	44	11.8	54	17.3
White Light (250 ft-c)	25	2.7	30	2.0	51	13.7	40	10.4
White Light (100 ft-c)	-	-	-	-	-	-	52	14.9
Red Light (100 ft-c)	27	2.6	40	7.0	58	17.3	-	-
Blue Light (100 ft-c)	-	-	-	-	-	-	55	12.6

TABLE 4. Physical measurements of A. americana cultures grown at 25 C in standard medium.

Illumination Conditions	Days of Incubation							
	4		5½		8		10	
	Dia. mm	Wt. mg	Dia. mm	Wt. mg	Dia. mm	Wt. mg	Dia. mm	Wt. mg
Darkness	54	18	66	25	89	26	90	24
White Light (250 ft-c)	48	14	56	23	89	24	89	23

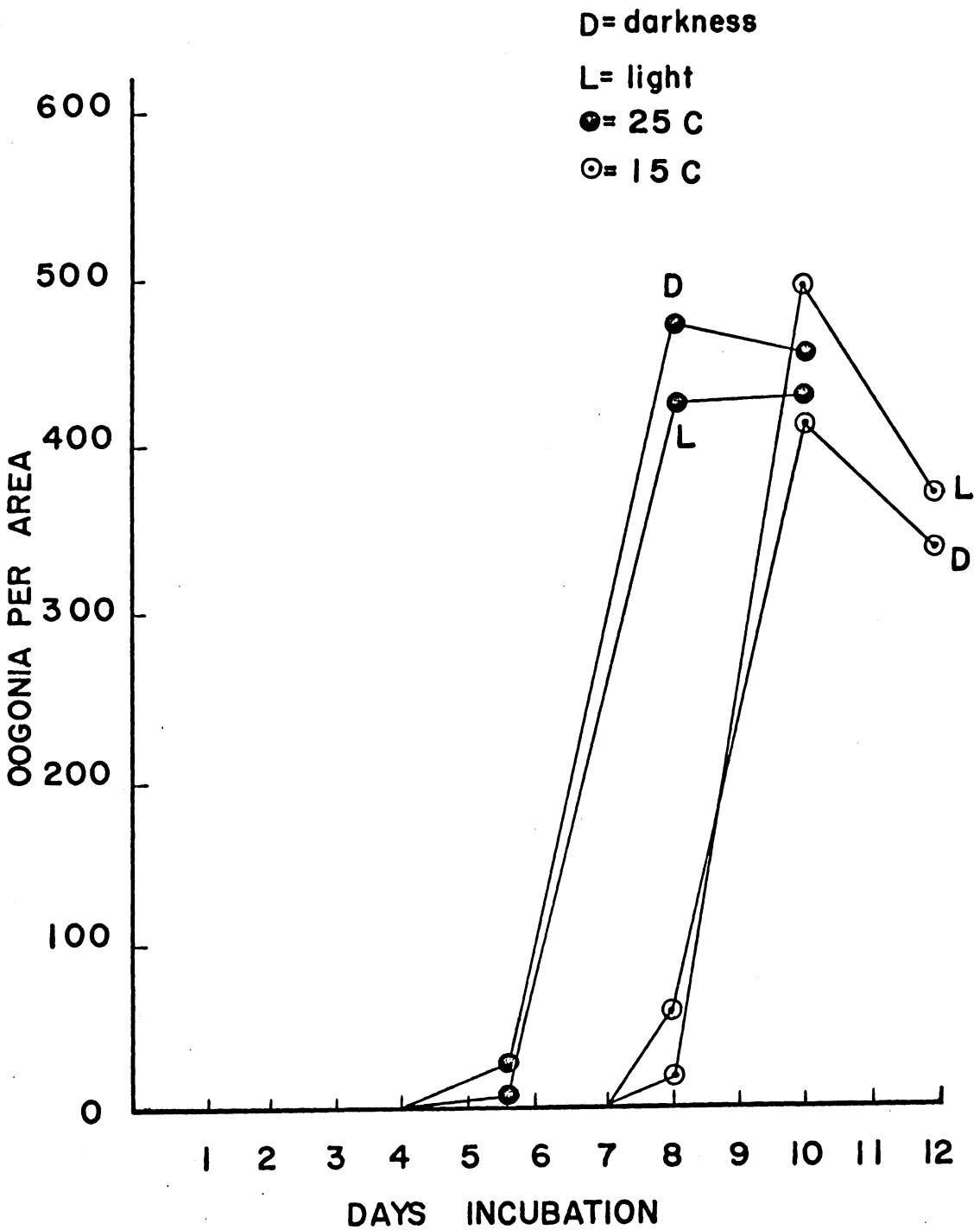


Fig. 11. Oogonia formed in A. americana in standard medium.

incubated in light had approximately one-half the number of oogonia found in the controls after seven days. After ten days the light series showed a higher peak of oogonia than did the controls at 15 C. At 25 C the controls reached peak oogonium formation after seven days and the light series reached its peak after about ten days incubation. Cultures exposed to red light of 100 ft-c intensity parallelled the darkness controls at seven, eight and ten days incubation. Table 3 shows that at 15 C the red light cultures were slightly larger than either light series or darkness controls. Cultures incubated in blue light (100 ft-c) were comparable to cultures incubated in white light (100 ft-c) after twelve days incubation (Table 3). After twelve days incubation at 15 C, cultures incubated in 250 ft-c white light averaged 364 oogonia per area; cultures incubated in darkness averaged 339 oogonia per area; white light (100 ft-c) gave 312 oogonia per area and those incubated in blue light (100 ft-c) averaged 292 oogonia per area. At 25 C the darkness controls showed more oogonia per area than cultures incubated in 25 ft-c light (Fig. 11).

The results of zoospore germination studies in A. americana are shown in Table 5. In light 8%

TABLE 5. Germination rates of A. americana zoospores at 25 C.

Illumination Conditions	Per Cent Germination	
	Hours Incubation	
	6	12
Darkness	23 %	12 %
White Light (100 ft-c)	8 %	12 %

germination took place after six hours incubation as opposed to 23 % germination in darkness. After twelve hours incubation the germination rates were equal although there was less total germination. Repeats gave similar results.

Saprolegnia parasitica:

Isolate 6-28-60 failed to fruit at any time during the experiments. Several combinations of light and temperature variations were tested with this isolate, all of which showed it to be indifferent to the illumination given. Fig. 12 shows a comparison of averages of four replicates at the end of seven and fourteen days incubation. Cultures exposed to two minutes ultraviolet light followed by incubation in 250 ft-c white light were inhibited slightly. Cultures exposed to ultraviolet light followed by incubation in darkness showed much inhibition of growth after seven days incubation. Photoreactivation was demonstrated here.

Isolate 2-27-59 did not fruit at any time during the experiments. This isolate showed a much lower growth rate than isolate 6-28-60 and also showed an inhibited growth rate in light (Fig. 12-13). This suggests a physiological strain difference in these two isolates.

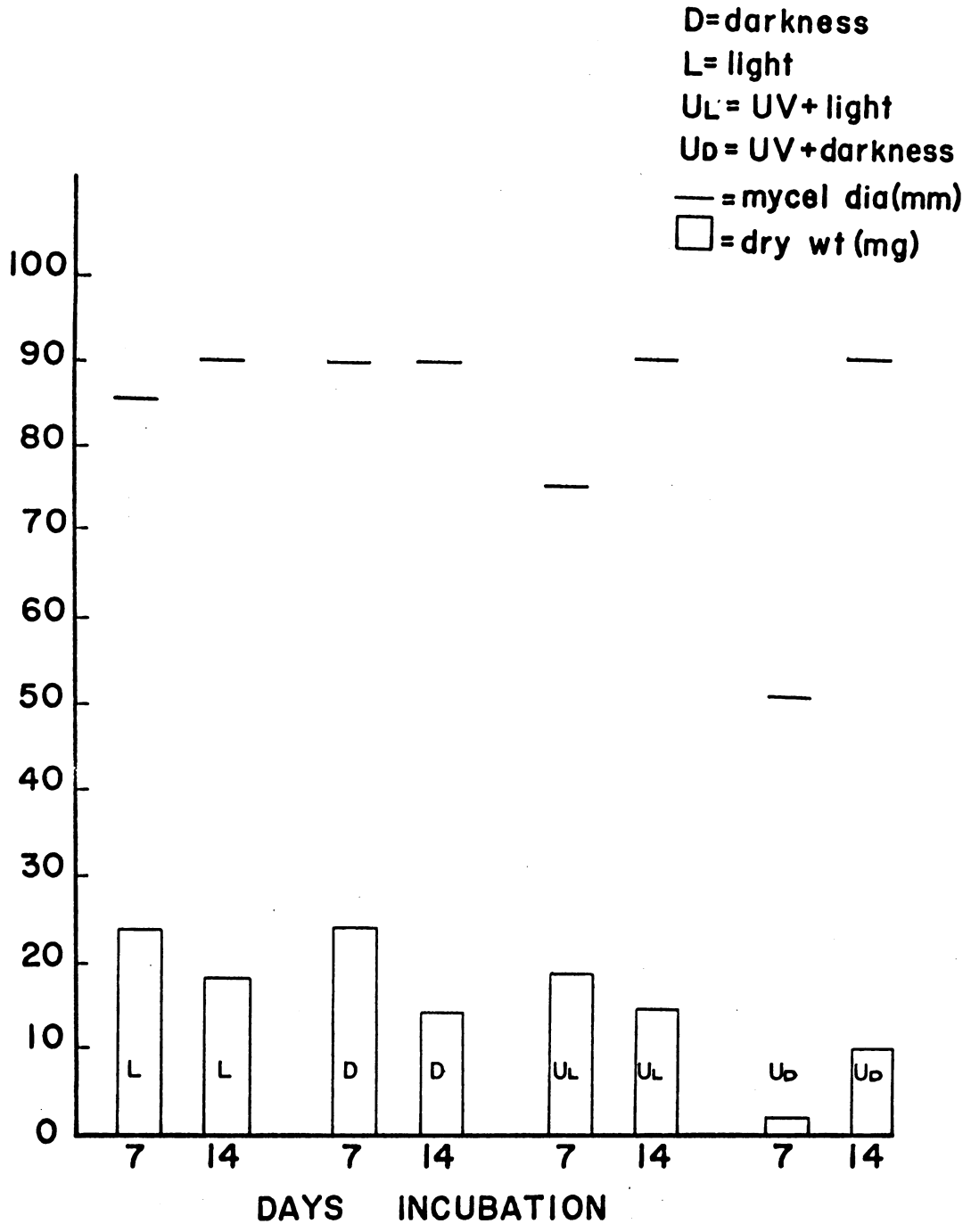


Fig. 12. S. parasitica (isolate 6-28-60) grown at 15 C.

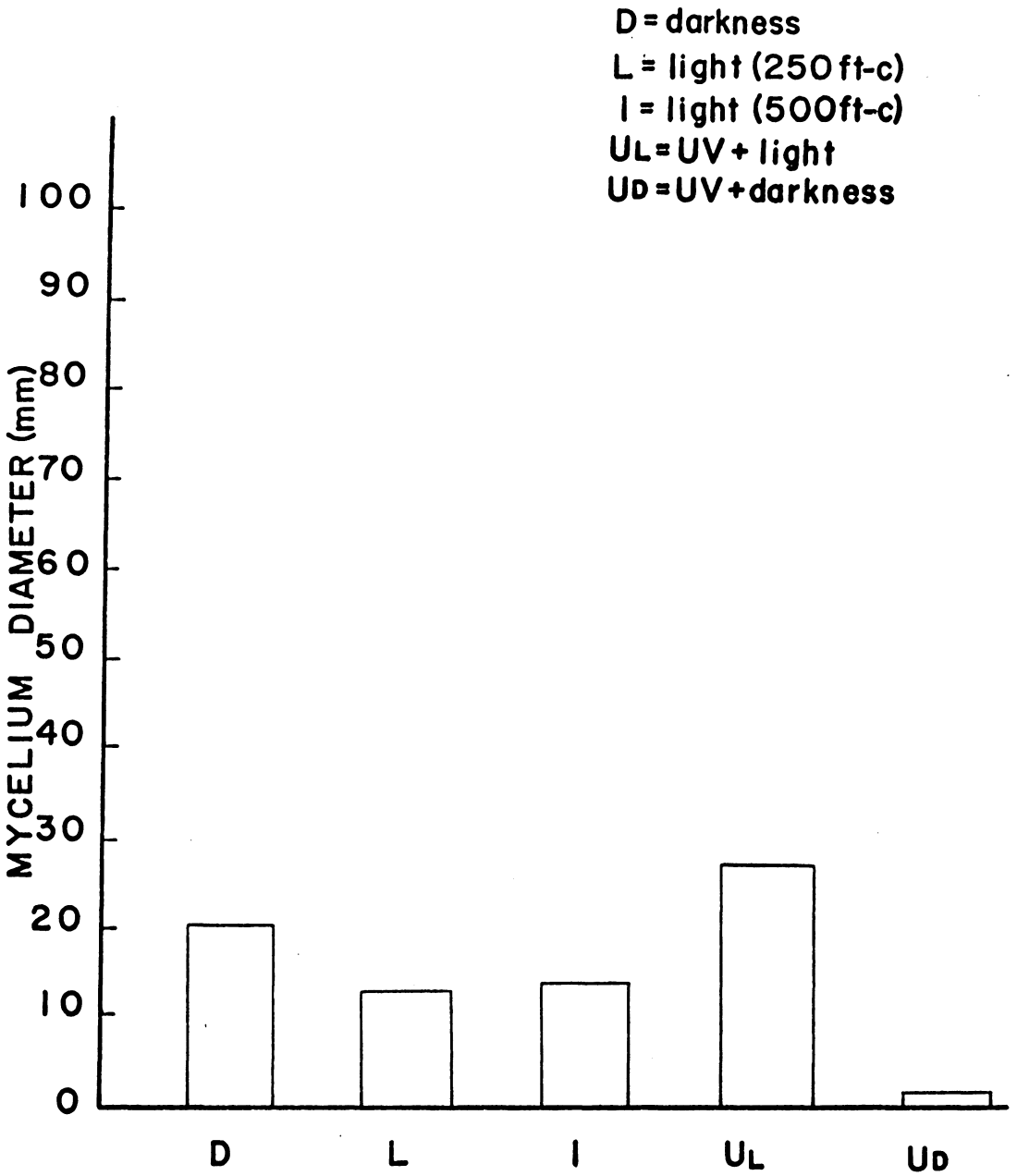


Fig. 13. S. parasitica (isolate 2-27-59) incubated at 15 C for seven days.

Those cultures exposed to two minutes ultraviolet light followed by incubation in light gave larger colony diameters, but this growth was atypical consisting of long unbranched hyphae. Cultures exposed to ultraviolet followed by incubation in darkness showed practically no growth after seven days. Photoreactivation was shown here.

The general pattern of oogonium formation was that oogonia formed first near the center of the colony with a gradual spreading of the oogonium formation as the diameter increased. Fig. 14 shows the distribution of oogonia per area on counts across the diameter of S. ferax cultures after seven, ten and twelve days incubation. This same pattern was found in A. americana.

The results to this point indicated that toxic substances were formed by light and that these substances in the medium resulted in the inhibition of growth and oogonium formation. It was found that when the inoculated medium was irradiated with two minutes ultraviolet light there was a definite inhibition of growth and oogonium formation. When the medium alone was irradiated, and then inoculated, both the vegetative growth and oogonium formation rates were increased (Table 6).

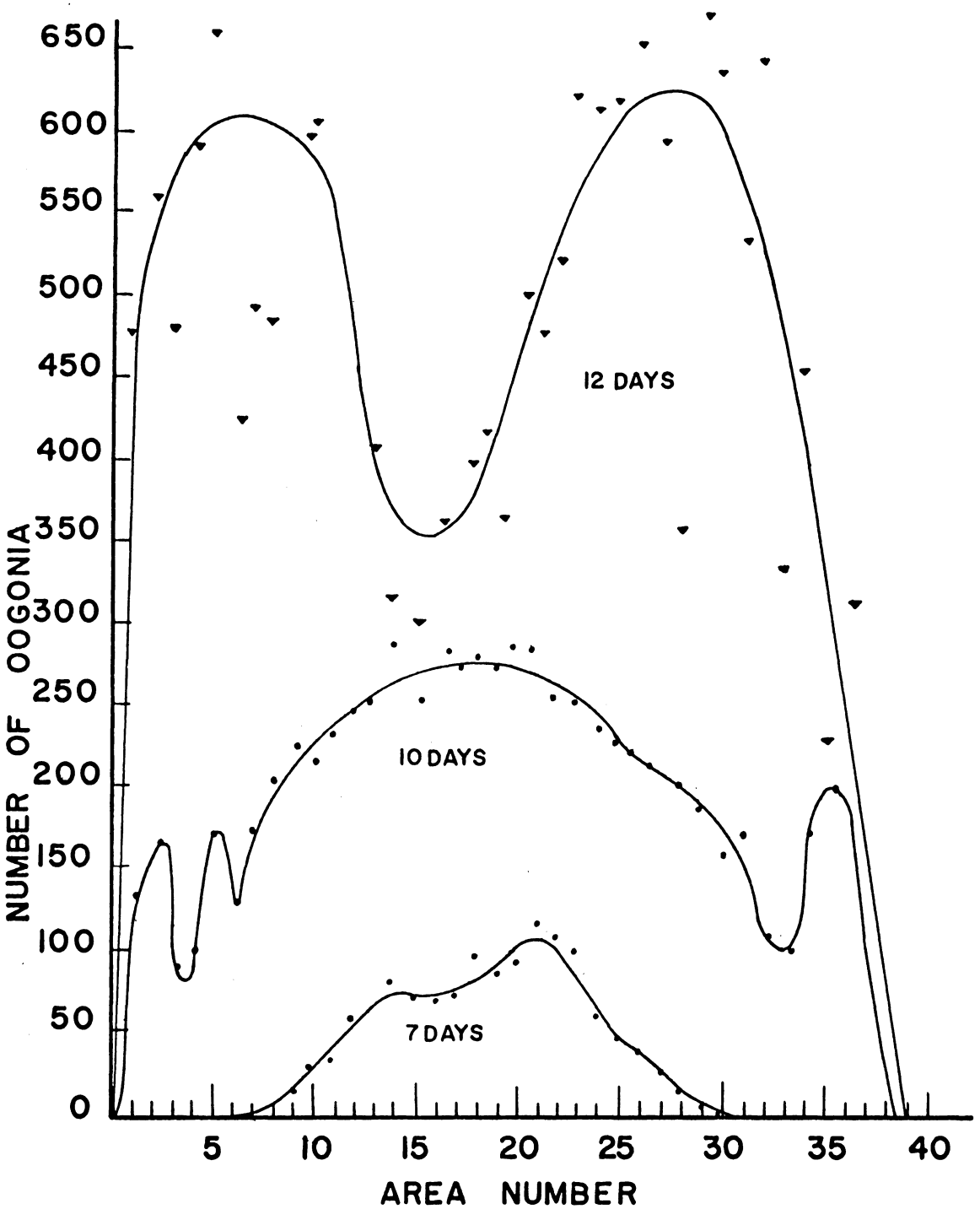


Fig. 14. Oogonium distribution pattern in typical S. ferax cultures.

TABLE 6. Averages of replicates of *S. ferax*[a] and *A. americana*[b] showing effects of ultraviolet irradiation.

Culture Conditions	Mycelium Dia., mm	Dry Wt. mg	Oogonia per Area	Days Incubation
No irradiation[a]	75	14	0	7
Medium irradiated AFTER inoculation[a]	70	13	0	7
Medium irradiated BEFORE inoculation[a]	84	18	3	7
Medium exposed to 7 days light prior to inoculation[b]	90	19	21	10
Medium held in darkness 7 days prior to inoculation[b]	90	19	126	10

In a second experiment, tubes of medium were exposed to 250 ft-c white light for seven days at 15 C prior to inoculation. Control tubes of medium were held in darkness for seven days at 15 C prior to inoculation. After ten days incubation at 15 C, the mycelium diameters and dry weights were the same for both sets but the darkness controls had produced six times as many oogonia as the light series (Table 6). A similar experiment was conducted with A. americana. Tubes of medium were exposed to light (250 ft-c) for 10 days at 15 C and controls were held in darkness for ten days at 15 C. The tubes were then autoclaved at 15 pounds pressure for 15 minutes, allowed to cool and then inoculated. After ten days incubation in darkness at 15 C the diameters and dry weights of both series were the same. The light-exposed medium produced colonies with an average of 21 oogonia per area while the darkness controls produced 126 oogonia per area (Table 6).

Standard medium which had been exposed to ten days light (250 ft-c) was tested for hydrogen peroxide using the blue peroxychromic acid test (Schumb, 1955). The test was negative but Schumb reported that it was dependable in showing hydrogen peroxide only at

concentrations of about ten parts per million and greater. A crude test based on catalase activity was tried. Medium was collected from cultures that had been exposed to ten days light (250 ft-c) and from cultures incubated in darkness, at 15 C. Five drops of dilute, commercial hydrogen peroxide solution were pipetted into each series of medium. The darkness series medium formed approximately twice the number of bubbles as the light series medium. This might indicate that cellular catalase had diffused into the darkness series medium as well as the light series, but the catalase had combined with peroxides or other substances formed in the light series medium.

Table 7 shows the results of a typical experiment with A. americana and shows the tremendous amount of variation found in the number of oogonia produced by replicates. In an attempt to discover if these variations were due to a genetic difference in the zoospores, S. ferax which also showed the same type of variation, was tested in an attempt to isolate any genetic segregants. Two cultures that showed few oogonia after ten days were allowed to form zoospores. Five replicate cultures were made using zoospores for each of the four colonies. After nine days incubation in darkness at 15 C,

TABLE 7. Replicates of A. americana incubated at 25 C showing extreme variation in rate of oogonium formation.

Replicate	Diameter mm	Dry Wt. mg	Total Count	Areas Counted	Oogonia per Area
Darkness Controls					
D-1	63	21.3	224	25	9
D-2	64	23.8	6	25	1
D-3	68	29.2	974	26	38
D-4	63	21.1	146	23	6
D-5	72	29.0	1128	27	42
Averages	66	24.9			20
White Light, 250 ft-c					
L-1	55	18.9	10	19	1
L-2	62	27.4	135	25	5
L-3	64	28.3	3	26	1
L-4	51	17.4	3	21	1
L-5	60	23.0	440	25	18
Averages	56	23			4

the cultures were examined and rated on the basis of no oogonia (-), few oogonia (+), many oogonia (+ +), and covered with oogonia (+ + +). Thirteen cultures fell into the (+ +) class, four cultures fell into the (+ + +) class, one fell into the (+) class, one fell into the (-) class and one contaminated culture was not counted. The (-) culture and one of the (+ + +) cultures were then allowed to form zoospores and four replicates were made from each culture. After nine days incubation in darkness at 15 C, these replicates were observed. All fell into the (+ + +) class.

To determine if a diffusable hormone were involved in the variation, water agar blocks (2.5 cm^2) were soaked for seven days in the medium removed from five fruiting cultures of S. ferax. Two of these agar blocks were placed in seven-day old cultures which had not begun to form oogonia. Two control agar blocks were placed in other non-fruiting cultures and two cultures were maintained without any agar blocks. After three days (ten days total incubation in darkness at 15 C) all the cultures were covered with oogonia. A previous experiment in which the medium from fruiting cultures of A. americana was exchanged with the medium from younger,

non-fruiting cultures showed that the spent medium increased the rate of oogonium formation slightly.

DISCUSSION

The general pattern found in this work was the inhibition of growth and sexual sporulation by light of all wave lengths tested. Two minutes exposure to ultraviolet light drastically reduced vegetative growth and oogonium formation rates; three minutes ultraviolet exposure was lethal. Red light apparently had less inhibitory effect on S. ferax than did white or blue light; red light showed little effect on A. americana. These results are similar to those reported by Etzhold (1960) who reported that in alternating light and darkness, oogonium formation in S. ferax was inhibited by filters and red cellophane which transmitted mainly red light. Etzhold theorized that in many cases, the effects of red light were due to contamination of red light by blue light. Vegetative growth of S. ferax was stimulated by light at 25 C (10 C above the optimum). Vegetative growth of A. americana and oogonium formation were stimulated by light at 15 C (10 C below the optimum). These data indicated that light might compensate for extreme temperature variations. Temperature differentials that compensated for loss of light have been reported by

Cochrane (1958). A. americana formed oogonia in light and darkness at 15 C and 25 C, although the rate of oogonium formation was lower at 15 C. S. ferax fruited profusely at 15 C in light and darkness but did not fruit at all at 25 C. Generally fungi are found to sporulate in a narrower temperature range than that which allows vegetative growth.

There are several reports concerning irradiated medium and its lethality and mutagenic qualities (Wyss, Stone and Clark, 1947; Wyss, et al., 1948; and Blank and Tiffney, 1936). Weinhold and Hendrix (1963) reported that several fungi were inhibited by nutrient agar that had been exposed to light from fluorescent tubes and incandescent bulbs. The inhibitive effect was found using potato-dextrose agar, potato-dextrose broth and V-8 Juice. No such inhibition was found using a semi-synthetic liquid medium. They also found that a duplication of the inhibition could be induced by adding hydrogen peroxide to the medium and negated by adding catalase. My work concerning irradiation of the medium with and without the inoculum indicated that in addition to the build-up of definitely toxic substances in the medium, ultraviolet exposure can cause stimulation. The stimulatory substances which were formed in the ultraviolet irradiated medium may actually

prove to be very low concentrations of the same toxic substances which showed inhibition. Very low concentrations of toxic substances have been shown to exhibit stimulatory effects in microorganisms.

Photoreactivation had not been reported for the fungi used in this work. Jagger (1958) stated that photoreactivation was typical of organisms that had been exposed to ultraviolet light followed by exposure to longer wave lengths. Photoreactivation was reported in bacteriophages, bacteria, fungi, Protozoans, insects and higher animals. Kelner (1952) reported that photoreactivation took place in dry Actinomycete spores which indicated that the reaction took place in the spore and not in the surrounding medium. The papers by Jagger (1958), Marmur et al. (1961) and Thimann (1963) cover the theoretical explanations for the mechanisms involved.

All values shown in this work were averages of replicates. One of the most interesting and perplexing findings of this research was variability in rates of oogonium formation. I believe that these variations merit further study. Some typical results are shown in Table 7. Similar variation was observed in

S. ferax. These extreme variations in replicates were found up to about twelve days incubation after which the oogonium counts stabilized in both fungi. Repeats of the experiments reflected similar variations. Mycelium diameters and dry weights were relatively consistent in the replicates but the number of oogonia produced varied tremendously. In nutritional studies concerning oogonium production in Achlya ambisexualis Raper, Barksdale (1962) stated:

> When glutamate was supplied at a concentration of 0.5 mg/ml (4.1 mg N per 100 ml), the range of variation for growth was 1.4 - 1.8 mg per ml (mean = 1.53), whereas oogonial production varied from 26 to 70 oogonia per field (mean = 46). Apparently some unknown factors having a greater effect on sexual reproduction than growth were inadequately controlled.

Evidently the same type of variable was not controlled in the present work. Checks were made of all experimental procedures in an attempt to discover any variable which might explain the results. Cork liners were removed from the test tubes. The blade used to cut out zoospores was changed; it was cooled thoroughly between inoculations. The medium was held in darkness at 15 C to prevent light or heat caused changes. The variation persisted.

The zoospores used for inoculum have classically been considered to be haploid and uninucleate. Staining

of the zoospores showed them to be uninucleate. If the zoospores were diploid (Sansome, 1963, and Raper, 1963) differences in the rates of oogonium formation could be explained on the basis of homozygous and heterozygous segregants. The results of the genetic experiments in attempts to isolate any of these segregants were too meagre to support any sweeping statements concerning the genetics of S. ferax. It was difficult to explain this variation on the basis of a nuclear gene if normal mitosis gave rise to all the nuclei produced by the single zoospore inoculum in each case. This research points to the need for further genetic studies of the Saprolegniaceae.

The experiments attempting to determine if a diffusable hormone were responsible for the variation indicated that in S. ferax: (1) any oogonium stimulating substance formed did not diffuse; (2) such substances were removed from the medium soon after oogonium formation; (3) break-down of the products occurred during the period in which the agar blocks were soaking, or (4) no such substances were produced. Further research is indicated in this area since Raper has demonstrated the hormonal control of fruiting in Achlya.

Since the vegetative growth of S. parasitica isolate 6-28-60 was not affected by light while isolate 2-27-59 was inhibited by light, and since these two isolates showed different growth responses and rates (Fig. 12-13) it was assumed that these isolates represent two physiological strains. The strain variations have been overlooked in most mycological works and too many reports of important research value have depended on the study of a single isolate.

It was interesting to note the oogonium distribution pattern shown in Fig. 14. This typical pattern was found in most of the cultures observed. It had been generally assumed that a bell-shaped curve would result when plotting oogonium distribution along the diameter of the colony, viz., more oogonia having formed near the center of the colony on the older hyphae. This pattern was found to be only partially true in the present study. After seven days incubation, an area near the center of the colony showed fewer oogonia than in the surrounding area. After ten days incubation, this area was less evident. After twelve days incubation the region was again very pronounced. Several explanations might account for this area of sparse oogonium formation. First, the

very small block of agar which originally encompassed the germinating zoospore occupied this area. Since the agar cube contained the standard medium, this might represent an area of slightly higher nutrient concentration and thus an area of oogonium inhibition. But if we were to take a typical colony at the end of seven, ten or twelve days growth period and make a section across the diameter of the colony and view this cross section, we would find that the section would physically, very closely approximate the shape of the curves shown in Fig. 14. Remembering that a colony would naturally form a sphere if allowed to grow in sufficiently deep medium, we can see that the petri dish caused a flattening of the sphere with the more branched hyphae surrounding a small crater formed by the few branches near the center of the colony. Since there are more branches surrounding the crater, there are more available hyphae on which the oogonia can form, thus the higher concentration of oogonia surrounding the crater. The young hyphae near the periphery have fewer oogonia since they are probably in contact with a more concentrated food supply. After twelve days, oogonia were found forming in the peripheral area also. This same type of zonal pattern was reported

for zoosporangium formation by Lee (1962) in Saprolegnia parasitica.

This research has pointed out several very important biological considerations. First of all the importance of the standardization of cultural techniques is definitely emphasized. By using two different media, light was shown to induce two entirely different responses. In the standard medium, inhibition of growth was found; in using the simple medium, stimulation of growth was found. A second important consideration is that of care in the interpretation of results. At first the apparent effect(s) of light were thought to be purely inhibitive with regard to the fungus; further research showed that in addition, the growth medium was changed by light and this in turn caused the inhibition. Another concept which was brought out was that of physiological strains and the importance of remembering that the data obtained on one isolate should not be considered as being representative of the species until a number of isolates have been examined. Finally the concept of "optimum" conditions was seen to be a relative term; what may be the optimum environmental condition for one response may not necessarily be the optimum condition for another response.

SUMMARY

1. Quantitative measurements of growth and oogonium production under varied conditions of illumination were made on the following fungi: (1) Saprolegnia ferax; (2) Achlya americana; (3) Two isolates of Saprolegnia parasitica. The fungi were grown in a chemically defined medium.

2. Light inhibited growth and oogonium formation rates in S. ferax and A. americana. Light inhibited zoospore germination in A. americana and growth in S. parasitica isolate 2-27-59. S. parasitica isolate 6-28-60 was not affected by light.

3. Differences in light reactions and growth rates of S. parasitica isolates indicated that they represent two physiological strains.

4. Photoreactivation was demonstrated in S. ferax and both isolates of S. parasitica.

5. Evidence was shown that light caused changes in the medium that inhibited vegetative growth and oogonium formation. The blue peroxychromic test for hydrogen peroxide was negative but apparent catalase activity was demonstrated.

6. Using a simple medium consisting of glucose, soluble starch and yeast extract, white light stimulated vegetative growth of S. ferax and A. americana.

7. A quantitative distributional pattern of oogonium formation was shown.

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ACKNOWLEDGEMENTS

I wish to express my thanks to the following members of my graduate committee: Dr. William W. Scott, my major professor, for his suggestions and guidance during the course of this study; Dr. Fred S. Orcutt for his help in obtaining financial assistance during my period of study; Dr. Stuart E. Neff, Dr. Robert E. Benoit, Dr. Maynard G. Hale and Dr. Kenneth A. Nicely offered many valuable aids and suggestions during the research.

Mr. Tom Evans, Department of Forestry and Wildlife, was very helpful with statistical considerations of the data. Dr. Duncan T. Patten, a former member of the Department of Biology, was very helpful in offering suggestions and allowing the use of much of his technical equipment.

I thank Dr. W. E. Chappell, Department of Plant Pathology and Physiology, for reviewing this manuscript and his participation as a member of my graduate committee.

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ABSTRACT

Pure cultures of Saprolegnia ferax (Gruith) Thuret, Achlya americana Humphrey, and two isolates of Saprolegnia parasitica Coker were grown in a chemically defined medium under controlled conditions of temperature and illumination. Light inhibited growth and oogonium formation rates in S. ferax and A. americana. Light inhibited zoospore germination rates in A. americana and the growth rate of S. parasitica isolate 2-27-59; S. parasitica isolate 6-28-60 was indifferent to light. Neither isolate of S. parasitica formed sexual structures during the experiments. Differences in light reactions and growth rates of these two isolates indicated physiological strain differences. Photoreactivation was demonstrated in S. ferax and both isolates of S. parasitica. Evidence was shown that light caused production of toxic substances in the medium which caused inhibition of growth and sporulation. The blue peroxychromic test for hydrogen peroxide in the medium was negative but apparent catalase activity was demonstrated. Using a simple medium consisting of glucose, soluble starch and yeast extract, white light stimulated vegetative growth in S. ferax and

A. americana. A quantitative distribution pattern of oogonium formation was shown.