THESIS

EFFECTSOFULTRA-VIOLET LIGHT ONVARIOUS FUNGI

Submitted by

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In partial fulfillment of the requirements for the Degree of Master of Science Colorado Agricultural College Fort Collins, Colorado

May 1, 1930

STATE AGRIGULT'L COLLEGE PORT COLLINS; COLO.

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	GRADUATE WORK
	<u>May 1</u> 193_
I HEREBY	RECOMMEND THAT THE THESIS PREPARED UND
MY SUPERVISION	BY_Elijah J. Starkey
ENTITLED EFFECT	S OF ULTRA-VIOLET LIGHT ON VARIOUS FUN
BE ACCEPTED AS I	FULFILLING THIS PART OF THE REQUIREMENT
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Approved by	Committee on Final Examination Committee on Advanced Degrees

This is to certify that Mr. E.J. Starkey has translated for me assigned passages of technical French bearing upon his graduate Botanical work.

Respectfully,

Head of Department of Modern Language.

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EFFECTS OF ULTRA-VIOLET LIGHT ON VARIOUS FUNGI

INTRODUCTION

Little work has been done on the effect of ultraviolet light on fungi, although considerable study has been given to the action of these radiations on green plants and on animals. The previous investigations which have been conducted along this line indicate that not all fungi give the same reaction to ultra-violet light. In order to have a better understanding of the general effects of these rays on fungi, the experiments presented in this thesis were conducted.

It is the purpose of this thesis to present data on the reaction of a large number of fungi in an effort to arrive at some possible correlation of the reaction of the various species to ultra-violet light. This involves a study of the effects of ultra-violet radiations on fungus sporulation, on changes of growth and metabolism.

The writer takes pleasure in acknowledging the encouragement and helpful criticism of Dr. L.W. Durrell who suggested the study here discussed. He also wishes to express his thanks to Mr. Edward Bodine, for his many helpful suggestions given in methods of technique, and his appreciations to Miss Mary F.Howe for her criticism of the manuscript.

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LITERATURE

Light is a very active agent in the lives of both plants and animals, whether its reaction be a stimulation or a retardation in life processes is dependent entirely on the individual.

Brefeld (5, 6 7) found in some species of <u>Coprinus</u> a complete suppression of fructification when plants were grown in darkness; in other species fructification took place but growth was slight. He also showed that the time required for exposure need not be very great, two or three hours, and the plant would develop normally though kept in darkness the rest of its growing period.

Elfving (13) sought to find the influence of light on metabolism. He used cultures of <u>Penicillium</u> spp. and a related fungue, (<u>Briaraea</u> sp.) growing in synthetic solution. Basing his conclusions on the dry weights obtained in the light and in the darkness, he decided that light acts as an inhibitor of organic synthesis, or that light restricts vegetative growth.

The most conclusive work offered is that of Terntz (21) who, working with <u>Ascophanus</u> carneus was able to produce asci only when plants were under the influence of light.

Chaudhuri (10) in his work with fungi states that

zonation is caused by the effects of illumination with ordinary daylight. Zonation appeared only on illuminated plates which proves this point. Hedgecock (14) found in experiments on zonation with <u>Cephalothecium</u> sp. that light was the determining factor. He showed that variation in temperature did not cause this zonation. He also found that light of different wave lengths had a different action on zone formation.

Visible light was used in the above experiments and it was found that its action was slow and hard to check, especially since some individuals were under observance for only a few hours. This handicap caused the investigator to search for some kind of rays which would give a faster reaction. Ultra-violet radiations were suggested for this purpose. These rays act more readily and produce chemical and structural changes in such a way that the action can be easily traced.

The first work done on the action of light of short wave length upon protoplasm was with bacteria and one-celled animals (amoeba and infusoria). Downes and Blunt (12) while using such organisms point out that the destructive action of the light upon protoplasm increases as the wave length decreases. Hertel (17) confirms the same work in a statement that the destructive action of light varies directly as the energy produced

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and inversely as the wave length. Bovie (3) while using Schumann rays noted that motile organisms (amoeba and infusoria) were stimulated by the action of these rays. First causing an increase in action, followed by a loss of power of coordination, and finally a disintegration of the living substance.

The main issue in the later work seems to be that of determining the wave lengths responsible for the abiotic effects of these rays. Browning and Russ (8) found the limits of germicidal action of ultra-violet light to be between the wave length of 2150-29600 Angstrom Units. Radiations having a wave length between 2960-3800 Angstrom Units, exhibited no killing effect upon bacteria which were treated. Bovie (3) found that radiations of a wave length shorter than 2925 Angstrom Units killed bacteria and spores of certain fungi in ten minutes, while radiations of 25 Angstrom Units lenger did not kill in two hours.

The exact action taken by these rays in causing an abiotic action to take place is not definitely known. Bovie (3) considers this phenomena due to the fact that light of shorter wave lengths (2800-2900 Angstrom Units) causes a coagulation of the protein within the protoplasm of the cell. The production of a visible coagulum or protein is due to two reactions, one

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that change caused by light, the other a temperature relation which is directly connected with this phenomena. Barr and Bovie (2) shows that the ultra-violet rays are strongly absorbed by the atomic groupings of the protoplasm, and the absorption of energy is accompanied by the atomic rearrangement due to chemical changes. The origin of protoplasm is one having its beginning at the colloidial interfaces, and it cannet be adequately considered without respect to time.

Henri (16) points out that the destructive action of the ultra-violet rays toward the life of an organism is almost exactly proportional to the coefficient of extinction of protoplasm; this proportionality indicates that the action of ultra-violet rays on microorganisms follows the law of photochemical adsorption, which has been found for the majority of photochemical reactions. This proportionality also indicates that the mechanism of the action of ultra-violet rays consists in a direct reaction on the cellular contents and not in an indirect action, such as the formation of H2O2 et. The rays of greatest destructive power only penetrate a few thousandths of a millimeter into the interior of the organism, and it is only for extremely small organisms that the action of these rays is analogous to a simple photochemical reaction.

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A killing effect does not always result when an organism is treated with ultra-violet light. Mme. Henri (15) exposed cultures of Bacillus anthracis in an aqueous solution to ultra-violet light, for periods varying from 1 to 40 minutes, and most bacteria were killed immediately. Few survived and many of these showed characteristics different from those of the typical Anthrax bacillus. Coccoid forms which remained stable during a period of two months - also thin filamentous forms not taking gram stains, nor liquefying gelatin, nor curdling milk, and producing an infection entirely different from anthrax inoculation. This culture remained absolutely fixed after daily sub-culturing for more than 80 days. Gram positive coccoid forms after passing thru an animal made their appearance (a part at least) as true type bacilli, at least approximating the typical anthrax type.

The latest work with the action of ultra-violet light has extended this study not only thru the bacteria but has included almost every kind of organism known. Pichler and Wober (19) successfully treated smutted wheat with ultra-violet and Rotogen rays. Stevens (20) while studying the effects of ultraviolet radiations on fungi noted that certain plates of <u>Glomerella cingulata</u> produced perithecia when treat-

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ed, while those which were not treated showed no sign of this stage. The culture under study was the result of a single spore isolation and could therefore be considered as non-sexual, providing such exists. The origin of the perithecia are visible two days after radiation as hyaline globose bodies and they can probably be detected at a much earlier date, since unusual branching occurs within a few hours after irradiation. All the strains of G. cingulata tested gave similar results. The activating region of the spectrum here used was found to lie between the wave lengths of 2760-3130 Angstrom Units. The effect is not the result of a chemical change in the media as produced by the radiations, but is a direct response of the mycelial cells to the radiations. The same effect is secured as would be expected from sexual forms.

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MATERIALS AND METHODS

Materials -

The experiments to date on the effect of ultraviolet light on fungi have been limited to a few genera, and no attempt has been made to summarize its action on various species. The writer has collected a large number of genera from many different families for this purpose. The source of the various genera has been rather variable, yet reliable. The following table shows sources and date of isolation of fungi used.

> Table 1. Species (strains) of the fungi used, their source and date of isolation.

Name of Organism	No.	Source Iso	lated
Absidia spinosa	20	Soil -Colorado	1928
Altenaria porii	81	Onion leaves-Rocky Ford	1929
Altenaria porii	18	Onion Leaves-Ft.Collins	1928
Altenaria solani	139	J.C.Gilman,F-2,Ames,Ia.	
Altenaria sp.	149	Sugar beet leaf-Ft.	
		Collins (Stewart)	1929
Altenaria sp.	80	Gladioli leaf- Ft.	
		Colling (LeClerg)	1928
Aspergillus cluvatus	11	E.L.LeClerg-Colo.soils	1927
Aspergillus flavus	14	E.L.LeClerg-Colo.soils	1927
Aspergillus fumigatus	7	E.L.LeClerg-Colo.soils	1927
Aspergillus glaucus	10	E.L.LeClerg-Colo.soils	1927
Aspergillus mentii	8	E.L.LeClerg-Colo.soils	1927
Aspergillus niger	13	E.L.LeClerg-Colo.soils	1927
Aspergillus ochroceus	16	E.L.LeClerg-Colo.soils	1927
Aspergillus terrens	9	E.L.LeClerg-Colo.soils	1927
Aspergillus sp.	15	E.L.LeClerg-Colo.soils	1927
Aspergillus sp.	65	E.L.LeClerg-Colo.soils	1927
Ascohyta pisi	128	J.C.Gilman, Ames, Ia.	1929
Botryosphaeria ribes	1	On apple -from Arlington	1928
Basisporium gallarum	52	J. C. Gilman	1928
Basisporium gallarum	138	J. C. Gilman F-25	1929
Botrytis sp.	44	Gladioli bearing sclerot	tia.
		from Drayton 12/11/28	1928
Botrytis sp.	151	Onion bulb, Ft.Colling	1929
Cephalothecium roseum	36	E.L.LeClerg-Colo. soil	

		- 8. (
Table 1 (continued)			
Name of Organism	No.	Source 1	solated
Cunninghamella verti-			
cellata	34	E.L.LeClerg-Colo.soil	
Cephalosporium acre-			
monium	142	J. C. Gilman F-2	1929
Cerastomella fimbrata	22	G. H. Elmer	1927
Chaetmonium obvaceum	120	J. C. Gilman F-40	1929
Coccomyces hiemalis	125	J. C. Gilman F-35	1929
Colletotrichum lagen-			
arum	3	Georgia - Stevens	1927
Colletotrichum linde-			1927
muthianum	51	0. H.Elmer, Manhattan, B	ans.
Colletotrichum pho-	2	O.H. Elmer, Manhattan	1927
moides		Kansas	
Cercospora sp.	82	D. Stewart, U. S. D. A.	. 19 29
		Ft.Collins and Arkanse	.8
		Valley	
Cercospora sp.	107	D. Stewart (2802)	1928
Cercospora sp.	108	D. Stewart (35-101)	1928
Cercospora sp.	105	D. Stewart (25-301)	1928
Cercospora sp.	109	D. Stewart (2801)	1928
Cercospora sp.	103	D. Stewart (35-102)	1928
Cercospora sp.	104	D. Stewart (2201)	1928
Cercospora sp.	106	D. Stewart (2202)	1928
Diplodia zeae	6	J. C. Gilman, Ames, Is	1928
Diplodia zeae	121	J. C. Gilman, F-42	1929
Discella populina	140	J. C.Gilman F-3	1958
Fusarium callistephii	80	J. C. Gilman	1920 1920
Fusarium culmorum	24	Christen	1928
Fusarium conglutinane	20	O. H. Elmer, Mannattar	1 1940
	00		10.00
Fusarium batatis	28	0. H.Elmer	1927
Fusarium sp.	02	Aster - Ft. Collins	196 9
Fusarium sp.	147	Aster root - Ft. Colli	1020
Fusarium sp.	00	Pine root - Denver	1000 1000
rusarium sp.	67	Union Build, FC.COILINE	1020
Fusarium sp.	1201	rea root - Denver	1696
Fusarium 11n1	127	J. C. GIIMan F-34	1090
Fusarium lycopersici	131	J. G. Gilman F-JA	1090
Fusarium niveum	190	J. C. GIIMAN BOLW	10.90
Fusarium orthoceras	90	Temel Dremair	上 ま ら ず
rusarium oxysporium	7 47	T A Cilmen T AZ	1090
	1/00	J. U. GIIMGH F-40 T. G. GiImge F 90	109 8
rusarium radicico11	_ 1.3 3	J. U. GIIMAN F-40	1345
Fusarium trichothecio	1	T () (113men T 90	10.98
ace	TTT	J. U. GILEAN F-42	1099
Fusarium sp.	23	Heuleulerg-volo-Bolls	1 1740
Flat White	29	Sterile mycellum 1801	1-
		ted irom culture (210	i ta ko

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	-10-		
Table 1 (continued)			
Name of Organism	No.	Source I	Bolated
Glomerella cingulata	33	Stevens (Lemon)Calif.	1927
Giberella saubinetti	54	O.H. Elmer (Wheat)	1927
Giberella saubinetti	L.	Barlev	1928
Gliobotrys albaviridis	49	O. H.Elmer	1927
Helminthosporium sativum	123	J. C. Gilman F-51	1929
Helminthosporium teres	144	J. C. Gilman F-52	1929
Hormodendron clados-			
noroides	33	Colorado soil	
Micor en.	87	D. Stewart	1098
Mucor erectum	126	$J_{\rm c}$ G Gilman $F_{\rm c}$ 45	1020
Mucor clearkilus	136	T. C. Gilman F-A6	1090
Mucor glomerule	145	F. T. Leflerg	1098
Micor Jougennengia	146	T Telland	1020
Wirens Lanschleists	197	T. C. Cilmon F. Kl	1020
Mangadha batanamanna Mangadha batanamanna	161	Ciloro _ T+ Calline	1020
Monascus neterosporus Maarogromium neregitigum	27	Braz aniona isolated	10.00
macrosporium parasicicum	orp	by F T Lollang Al	1760
		by he he heurerge All	30) # 1 m m
		D harbarum anarea	rerug .
Daniaillian communi	A 15	F. nervarum spores.	1008
Penicillium eitmi num	40	U. H.BIMER	TAUL
renicillium citri num	46	E. L. Deulerg-0010.	10.08
Denici 17 inne shares some	A 84		TASt
reniciliium chrysogenum	47	E. L. Leulerg-Colo.	1000
Band all the same and an	7.0		1927
renicillium expansum	38	U. H.Elmer	1927
Penicillium glacioli	39	U.H.Elmer	1927
renicillium purpurogenum	20	E. L. Leclerg-Colo.	1000
		BOLL	1927
Penicillium stoloniierum	46	E. L. Leulerg-Colo.	
-		8011	1927
Penicillium viridicatum	43	E. L.LeClerg-Colo.	
		8011	1927
Phoma betae (77)	94	D. Stewart-sugar beet	
		(77)	1928
Phoma betae (75)	92	D. Stewart	1928
rnoma betae (122)	90	D. Stewart	1928
Phoma betae (71)	93	D. Stewart	1928
Phoma betae (70)	97	D.Stewart	1928
Phoma betae (68)	98	D. Stewart	1928
Phoma betae (76)	101	D. Stewart	1928
Phoma betae (80)	91	D. Stewart	1928
Phoma betae (72)	96	D. Stewart	1928
Phoma sp.	148	D. Stewart	1928
Phoma betae (217)	32	Unknown	
Phoma sp.	59	Aster root - Ft. Coll	Lins
	··· •		1929
Phoma lingham	118	J. C. Gilman F-22	1929
Physalospora cydoniae	48	O. H.Elmer	1927
nysarospora cydoniae	40	V• fi•Fither	1.745E

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Table 1 (continued) Name of Organism	No.	Source	[solated
Physalospora cydonae	143	J. C. Gilman F-18	1929
Physalospora fusca	17	Stevens	1927
Plactodiscellae veneta	115	J. C. Gilman F-36	1929
Pythium sp.	83	D. Stewart-sugar be	et
		-	1928
Rhizoctonia crocorum	95	D. Stewart	1929
Rhizoctonia solani	102	Solanu tuberosum-	
		Greeley	1929
Rhizoctonia sp.	100	Sugar beet	1929
Rhizoctonia sp.	84	Solanu Jamesii-	
		Boulder	1929
Rhizoctonia sp.	30	Sugar beet-E.L.	
		LeClerg	1928
Rhizopus nigricans	26	Amer. type culture	E
		1200/	1927
Rhizopus nigricans	27	Amer. type culture	
		1201-	1927
Sclerotium bataticola	5	O. H. Elmer	1927
Sclerotium gladioli	117	J. C. Gilman F-15	1929
Sclerotium delphinii	136	J.C. Gilman F-30	1929
Sclerotium intermedia	112	J. C. Gilman F-23	1929
Sclerotium racini	122	J.C. Gilman F-26	1929
Sclerotinia american a	124	J. C.Gilman F-49	1929
Sclerotinia minor	114	J. C.Gilman F-50	1929
Septoria gladioli(Can.)	132	J. C. Gilman F-16	1929
Septoria lycopersici	116	J. C. Gilman F-21	1929
Spores 216	31	Unknown	
Trichoderma lignorum	55	Unknown	
Ustilago zeae (Iowa)	110	J. C. Gilman F-11	1929
Ustilago zeae (Wis.)	113	J. C. Gilman F-9	1929
Verticillium albo atrum	133	J. C. Gilman F-53	1929
Verticillium glaucum	152	Gardenia - Denver	1930

In some instances the source and date of isolation has not been given, due to incomplete records. These cultures were obtained from previous collections.

Methods -

The media used in the study of the effects of ultra-violet radiation upon fungi were as follows: potato dextrose agar, oatmeal agar, cornmeal agar, cytime agar "a" and "b", tyrosine agar, "a" and "b", peptone dextrose agar, and malt solution.

The potato dextrose agar was prepared by the usual methods used in the plant pathological laboratories.

The oatmaal agar was prepared by adding 60 gramm of finely ground rolled oats to one liter of water. This mixture was warmed gently in a double boiler for 10 to 15 minutes, making a rather thin gruel. Twenty grams of finely cut agar was stirred in at this stage and the heat held constant until it was all dissolved; distilled water was then added to restore the original volume. The material was then placed in flasks and sterilized in an autoclave at 10 pounds pressure for 40 minutes. Fractional sterilization gave very good results with this media (15 minute sterilization for three successive days).

Cystine agar "a" was prepared by first making up an ordinary non nutrient agar (50 grams of agar to 1000cc. of distilled water). Then cystine was added at the rate of 4 grams per 1000cc. nonnutrient agar. It was then placed in an autoclave and cooked for one hour at 12 to 15 pounds pressure, filtered, poured into flasks and sterilized in an autoclave at not over 12 pounds pressure for 40 minutes.

Cystine agar "b" was prepared very similar to "a"

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except that there was an alcoholic chlorophyll extract added and 20 cc. of chlorophyll extract was added per 1000 cc. of cystine *a* agar. (Chlorophyll extract was made by grinding 10 grams of geranium leaves very thoroughly and then adding 50 cc. of 95% alcohol to remove the chlorophyll.) The media was then poured in flasks, sterilized in an autoclave at 7-9 pounds pressure for 40 minutes.

Tyrosine agars were made by a similar method, the only difference being that tyrosine was used instead of cysting.

Peptone dextrose agar was prepared by adding a StandardDunham's Solution (distilled water 1000cc.) to 1000cc. of non-nutrient agar agar, then adding 40 grams dextrose (2 per cent). The media was then treated the same as potato dextrose agar.

Malt solution was prepared by adding 50 grams of malt extract to one liter of water. The solution was then placed in test tubes and sterilized in an autoclave at 15 pounds pressure for 30 minutes.

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METHODS AND PROCEDURE

Cultural methods

The various organisms used were all treated under similar conditions. Stock cultures were maintained at room temperature, and from these transfers were made to poured sterile petri dishes. Plates were run in three definite series. I. Plates which were placed immediately in the dark and kept there throughout the experiment. 2. Plates exposed to ordinary daylight for the entire period. 3.Plates which were placed immediately in the dark and later (3 to 5 days) exposed for various periods to the action of ultra-violet radiations. All the above plates were kept at a constant temperature throughout the experiment. Room temperature was about 22°C. while the incubator was run at 24°C. in order to prevent a fluctuation.

In some cases it was found necessary to use single spore isolations. These were made in all the cases where the organism produced spores. It was accomplished by making a very dilute spore suspension and then streaking a poured agar plate with a sterile loop which had been dipped in the suspension. The plate was watched and when the spores began to germinate, the individual spores were removed with small bits of agar to other sterile petri plates. Methods of treatment of cultures

The colonies were placed under their respective conditions and allowed to grow for a period of time. The rate of growth of the colonies varied considerable, so that not all colonies inoculated at the same time were ready to treat together. Colonies were allowed to reach about one inch in diameter before treatment, except in the case of very slow growers which were treated at about onehalf inch size. Plates to be treated were marked and set aside so that their period of exposure would be known at the time of irradiation.

The surroundings were then washed thoroughly with a l:1000 solution of Mercuric Chloride so that all free organisms would be destroyed and the possibilities of contamination would be removed. The lids were then removed from the plates and they were placed two or three at a time under a Cooper Hewitt Mercury Vapor Lamp No. DC-6HDI for periods varying from two seconds to four minutes, and at distances ranging from 20 to 60 centimeters. The final distance used in all experiments was 30.5 centimeters, and the time was 30 seconds.

Method of media treatment

Cystine and tyrosine agars were prepared as explained in the fore part of this paper, and previous to the time of inoculation of the cultures the plates were placed

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under the radiations of the ultra-violet light for a two hour period. The purpose being to see if any action would take place other than what is normally noticed in the short radiations. Bailey (1) states that there are no appreciable effects of ultra-violet radiations on media when it is exposed for periods less than two hours. The plates were placed in a sterile container on a slab of ice so that the temperature could be kept down. If no care was used to keep down the heat then a drying out of the media occurred. Plates placed at 30 cms. from the mercury tube reached a temperature of as high as 78°C. in less than three minutes. Figure 1 shows the change in temperature over various periods of exposure. These curves show the average rise in temperature for a large number of trials. This shows the value of keeping the temperature as low as possible. Potato dextrose agar was also treated but no appreciable change could be noted in the growth of the organism on these plates and on the plates that were not radiated.

Method of growth studies

Plates containing potato-dextrose agar were inoculated with mycelium of <u>Mucor</u> sp. No. 87. Growth was allowed to continue for 36-48 hours before the study began. The tops were removed from the plates and they were then placed on a microscope stage and made stationary. Little

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care needed to be exercised here in order to prevent contamination, since the plates were only used for a few hours The ultra-violet light was then fixed in such a manner that it would produce only a beam of light. This was accomplished by using a series of slits placed at right angles to each other. The distance from the source of light was 35 cms. A focus was made on the growing point of one filament of the hyphae and its rate of growth observed for a period before exposure to ultra-violet light was made. Readings were made at 15 minute intervals to check the rate of growth and make possible the plotting of a curve to show the exact effects of the light.

Method of using filters

Filters were used in order that a certain wave length of light would be transmitted. Two filters were used which had a wide range of transmission. The intensity of the light is greatly decreased when a filter is used rather than the open arc, therefore the culture must be allowed to remain in the light for a longer period of time in order to get the same results as a short exposure in the open arc. The time allowed for the exposure of cultures under the two filters was taken as two minutes. The longer period of exposure made it necessary to make some method whereby contamination could be kept down. This was accomplished by removing the top from the plate

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and placing the filter down on the surface of the plate. The filter was dipped in mercuric chloride previous to the time of exposure. When the plate had been exposed for the correct time the filter was removed and the top placed on the plate.

The filters used were filter A586 furnished by the Cooper Hewitt Company, the other a red-purple glass filter 3.29mm. thick which was furnished by the Amersil Company Inc. The transmission curves for the two filters are shown in Figure 2 as furnished by the companies manufacturing these filters.

EXPERIMENTAL DATA

A generalization of the action of ultra-violet light on fungi has not yet been made. Some genera have been tested quite thoroughly, yet no attempt has been made to summarize the results. The work which has been conducted in this laboratory has paralled the work of other authors where the same species have been studied.

Table 2 gives a summary of the light reaction of over one hundred species of fungi. There will also be offered a summary of several strains of the same species which will show the variation that may be expected within a known group.

In the accompanying table (No. 2) it may be noted that all but 35 species of fungi tested produced spores

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TABLE 2 SUMMARY OF REACTIONS OF VARIOUS FUNGI TO DIFFERENT KINDS OF LIGHT

opposite 20

Name of Organism	No. of	<u> </u>	Spores		D.	L.V.I	Ring f	ormatio rk	n U.\	/.L.	Pe	ritheci	a	<u>`</u> 1	Pycnidia	L		Sclero I	tia	Colony	Color
	Colony	D.I.	Dark U	.V.L.	Sp.	My.	Sp.	My.	Sp.	My.	D.L.	Dark	U.V.L.	D.L.	Dark	U.V.L.	D.L.	Dark	U.V.L.	flat'nd	Change
Absidia spinosa Altenaria porii (1929)	20 81		++	+		-	-	-	-	-	•	-	- / Ps	-	-	-	-	-	-	+	
Altenaria porii (1928) Altenaria solani	18 139	77	7	7	-	-	-	-	-	7			/ Ps	-	-	-		-	-	+	<u>+</u>
Altenaria sp. (Sugar beet) Altenaria sp. (Gladiola Leaf)	149 80	<i>7</i>		t	-	-	-	-	-	+			-	-	-	-	-	-	-	7	
Aspergillus cluvatus Aspergillus flavus	<u>11</u> 14	F	7	7	7	-	-	-	7	-			-	-		-	-	-	-		-
Aspergillus fumigatus Aspergillus glaucus	7	1	4	+	-	-		-	7	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus mentii Aspergillus niger	8 13	- 7	7	7	- <u>+</u>	-	-	-	<i>†</i>	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus ochroceus Aspergillus terrens	16 9	7	7	7 7	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus sp. Aspergillus sp.	15 65	7		7	- 4-		-	-	7	-	-	-	-	-	-	-		-	-	-	-
Ascohyta pisi Botryosphaeria ribes	128 1	-		<u>+</u>		-	-	-			-		-	-	-	-	-	-	-		-
Basisporium gallarum Basisporium gallarum (Iowa)	<u>52</u> 138	4	7	/ /	-	-	-	-	-	<i>+</i> <i>+</i>		-	-	-	-	-	-	-		- 	-
Botrytis sp. (Onion) Botrytis sp. (Gladioli)	<u>151</u> 44	7	7	ŧ.	-	-	-	-	- <u></u>	Asc.	-		-	-	-	-		-			-
Cephalothecium roseum Cunninghamella verticellata	<u>36</u> 34	17	17	7 7		-	-	-	-		-	-	-	-	-	-		-	-	- <u>+</u>	-
Cephalosporium acremonium Cerastomella fimbrata	142	- F	17	Ŧ	Ŧ	-	-	-	-	<u></u>	-	-	-	-	-	-	-	-			-
Chaetmonium obvaceum Coccomycesthiemalis	120 125	- <u>+</u>				-	-	-		Ż	-	-	- F Ps	-	-	-		-	-		
Colletotrichium lindemuthianum Colletotrichium lagenarum	<u>51</u> 3		++	Ŧ	- '		-	-	-	-	-	-		-	-		-	-	-		
Colletotrichium phomoides Cercospora sp. (Stewart)	2 82			<u> </u>	-		-		-	-	-		-	-	-	-	-	-	-		
Cercospora sp. (2802) Cercospora sp. (35-101)	107 108			?	-	-	-	-		- 7	-		-	-	-	-	-	-	-		Ŧ
Cercospora sp. (25-301) Cercospora sp. (2801)	105 109		=	7	-		-	-	-	7	-		-		-	-	-	-	-	-1	
Cercospora sp. (35-102) Cercospora sp. (2201)	103 104				-	-	-	-	-	-7-	-	-	-	-	-	-	-	-	-	- +	7
<u>Cercospora sp. (2202)</u> Diplodia zeae	106 6	Ē		E_	-	-	-	-	-	7	-	-	-	-	-		-	-	-		-
Diplodia zeae (Iowa) Discella populina	121 140	Ŧ	Ŧ	Ŧ	-		-			1	-		-	-	-		-	-	-	7	<u>_</u>
<u>Fusarium callistephii</u> Fusarium culmorum	85 24	+		Ŧ		-	-	-	-	-+	-	-	-	-	-	-	-	-	-	- 7/	-
<u>Fusarium conglutinans</u> Fusarium batatis	25 28	+	-4	4		-	-	-	-	-	-	-	-	-	-	-	-	-	-	<u></u>	-
Fusarium (Aster) Fusarium (Aster root)	62 147	F	7	7		-	-	-	-		-	-	-	-	-	-	-	-	-	+	-
Fusarium (Pine) Fusarium (Dahlia)	<u>88</u> 60	17	+++++++++++++++++++++++++++++++++++++++	7		-	-	-	-	-7-	-	-	-	-	-	-	-	-	-		
Fusarium (Onion) Fusarium lini	57 137	<u>+</u>	7	-	-			-		- t	-	-	-	-	-	-	-	-	-	7	
Fusarium lycopersici. Fusarium niveum	$\frac{131}{130}$		-	-	-	-		-		-4-	-		-		-		-	-	-	<i>†</i> <i>†</i>	
Fusarium orthoceras Fusarium oxysporium gladioli	86 141			<u> </u>	-	-	-			-7-	-		-	-	-	-	<u> </u>	-		7	-
Fusarium radicicola Fusarium tricothecoides	129 111	7	-7	4	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	4	-
Fusarium sp. Flat White (216 spores)	23 29			7		-	-	-	-	-{		-		-	-		-	-		- <u>†</u>	-
<u>Glomerella Cingulata</u> Giberella saubinetii (wheat)	<u>33</u> 554	7	7	Ź	-	-	-	-	-		-	-	-	<u></u>			-	-	-	7	-
<u>Giberella Saubinetii (Barley)</u> Gliobotrys albaviridis	49	1-2-	7	ŧ	7		-	-			-	-	-	-	-		-	-		- <u>†</u>	
Helminthosporium sativum Helminthosporium teres	123 144	7	17	Ź		-					-		-	-	-		-			7	-
Hormodendron cladosporoides Mucor sp. (Stewart)	35 87	+ 7-	+ +	7-		-		-		ZKn		-		-	-	-		-	-	-	
Mucor erectum Mucor gleophilus	$\frac{126}{135}$	1-7-	1	ź		-	-		-	-	-		-	-	-	-	-	-		-	
Mucor glomerula Mucor lausannensis	$\frac{145}{146}$	1/2		<u>t</u>	-	-	-	-	-		-		-	-	-	-	-	-		-	
Mucor racemosus Macrosporium parasiticum	127 81p	-2-	- /	ŧ Ŧ	-	-	-	-	-	- 	-	-	Ŧ	-	-	-	-	-		7	
Penicillium communi Penicillium citrinum	45 42	17	1 F	7 7	-		-	-	7V 7	-	-	-	-	-	-	-	-	-		-	
Penicillium chrysogenum Penicillium expansum	47 38	17	7	t	-		-	-	<i>†</i> <i>†</i>		-	-	-	-	-	-	-	-	-		
Penicillium gladioli Penicillium purpuragenum	<u>39</u> 50		1 Z	ŧ.	-	-	-		<i>†</i> <i>†</i>		-		-	-	-	-		-	-	-	-
Penicillium stoloniferum Penicillium viridicatum	46 43	-7-	7-	7		-	-		- <i>†</i> - <i>7</i>	-	-	-	-	-	-	-		-	-	-	
Rhoma betae (77) Rhoma betae (75	94	Ť	7		-			-	-							- 1					
Phoma betae (122) Phoma betae (71)	90 93	-			-	-				7	-	-							-	- 7	
Phoma betae (70) Phoma betae (68)	97 98	Ž	17	2					-	7	-		-	Ē	7	-7/-	-	-	-	1/	Ž
Phoma betae (76) Phoma betae (80)	101 91	Ź		7	-	-			-	Ē.	-			Ŧ		Ē	-	-	-	- -	- F
Phoma betae (72) Phoma betae sp.	196	1 - ź		- *	-	-	-		-	7	-					- <i>t</i>	-	-			-
Phoma (Aster root)	59	- Ŧ	Ŧ	7		-	-	-	-	<i>t</i>			-	+	7	7PS 7	-	-		- F 	
Phoma lingham Physalospora cydoniae	48			-	-				-	7	-		-	-	-	/Ps	-	-	-	- 7	
Rhysalospora cydoniae (lowa) Rhysalospora fusca	140	Ż		7		-		-		7	-		-	12	-	4	-	-	-		-
Pythium (Sugar beet)	83	7	7	7		-			-	- IV.n	-	-	-	-	-		-	-	-		-
Rhizoctonia Solani Rhizoctonia (Subar heat)	102			-	-	-			-	-fSc	-	-	-	-	-	-	+	7	ŧ,		-
Rhizoctonia (Sugar beet) (L.C.)	30				-	-		-	-	-Kn			-							<u> </u>	-
Rhizo, us nigricans (1200)	26	Ŧ	- F	7	-			-				-	-	-	-			-	-		-
Sclerotium bataticola	5	- 7		7	-	-			-	+	-					-	-t,-				-
Sclerctium intermedia	136				-	-			-		-	-	-			-		Ŧ	-+		-
Sclerotinia americana	124			-	-				-	750 750	-		-		-	-	Ŧ	-			-
Septoria gladioli (Can.)	1.14	Ż		Ē		-	-	-	-		-				-	-		-	-	-	+
Septoria lycopersici Spores (216)	<u>116</u> <u>31</u>	7	7		- 		-		Ż	-	-	-				-	-		-	-	
Ustilago zeae (Iowa)	<u>55</u> 110	7	17	7	-		-		-	7		-			-		-	-	-		-
Verticillium alba-atrum	$\frac{113}{133}$	1	1		-		-		-	Ž	-	=	-	-					-	7	-
Verticillium sp. (Gardenia)	152	17	1			<u> </u>		-	<u>/</u>		L <u>-</u>			<u> </u>		L	<u> </u>	1_ <u>t</u>	<u> </u>	L	<u> </u>



in daylight, and all but 38 produced spores in darkness. Ultra-violet radiations caused spore production in all but 28 species.

Spore ring formation is rather common in cultures allowed to remain in daylight, 17 species were found to produce rings under these conditions, while no cultures were found to produce true rings in complete darkness. Twenty-seven species formed true rings in irradiated plates with ultra-violet light. Figure 3 shows a representative spore ring formation, this ring was formed under daylight conditions.

Mycelial ring formation is entirely different from spore rings, and seems to be due to a flattening of the mycelial mass. Only one species produced this type ring in daylight, none in darkness, but 65 species subjected to ultra-violet light showed the phenomena.

No species were found to produce perithecia in either daylight or darkness, yet four species produced such in irradiated plates.

Nine species produced pycnidia in daylight, six in darkness and sixteen in treated plates.

Sclerotial production seems to be favored by some kinds of light, since 10 species produced sclerotia in daylight, four in darkness and 11 in plates given ultraviolet light treatments.

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Fig. 3. The above photograph represents typical daylight rings as developed on an untreated plate of Culture 216.



Sixty-eight species showed a flattening of the mycelial mass by ultra-violet radiations, and a color change due to irradiation was noted in 23 species.

The above summary gives an estimate of the gross differences in reaction which we may percieve due to the various treatments.

It will be noted in Table 2 there is deviation from the expected results in some cases, this deviation is shown by sublettering.

Kn - a knotting of the mycelium, yet not forming sclerotia. It is characteristic of <u>R</u>. <u>crocorum</u> and some species of <u>Mucor</u>.

Ps = pseudo pycnidial or perithecial development. knots of mycelium resembling pycnydia or perithecia, yet no spores are formed.

Sc = sclerotia, ordinarily flattened mycelial rings are different from this type of heavy sclerotial ring.

x - see special notes on <u>Fusarium</u> for type of fruiting caused by radiations.

The above table (No. 2) shows that most of all species of the same genus react similarily to ultraviolet radiations. Whether this action be as a retardation or apparent stimulation.

The above table is voluminous therefore the experimental work which comprises the table will be shown under four separate heads:

The effects of ultra-violet light (1) on sporulation; (2) on growth; (3) on protoplasmic movements and (4) on metabolism.

THE EFFECT OF ULTRA-VIOLET RADIATIONS

ON THE SPORULATION OF FUNGI.

The data presented by other workers to date has shown that there is an increase of sporulation in some species of fungi when they are subjected to the radiations of light of short wave lengths. In the accompanying data it may be seen that this apparent stimulation may take the form of causing the perfect stage of a fungus to be produced as in the case of <u>Macrosporium parasiticum</u>, or it may cause the production of pycnidia in apparently sterile cultures as in <u>Phoma</u>, or even in the increased conidial production as found in the case of <u>Penicillium</u> and other fungi.

The process of sporulation is one whose cause is not definitely known at the present time. Coons (11) suggested the theory that at any time after proper mycelial growth has taken place that sporulation can be brought about. The means necessary to cause this action to take place depends upon the organism under question. The chief cause being that some circumstance arises whereby the conditions were not correct for mycelial

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growth. He suggested light, heat, and food as being possible causes of the phenomenon.

In the experiments presented in the following discussion there have been tested out two of the above mentioned causes, light and temperature.

It was found that various genera show a difference in reaction to ultra-violet radiations. For instance, the effect on the sporulation of <u>Fusarium</u> sp. was different than that of Rhizoctonia; for this reason, the more important reactive genera will be discussed separately. The first to be treated will be certain species of the genus <u>Fusarium</u>.

FUSARIUM

The species of the genus <u>Fusarium</u> studied react with a great degree of constancy toward ultra-violet radiations. The chief reaction being that macrospores are produced in great abundance in most treated cultures while the checks show few to no macrospores. Microspore production was apparently unchanged.

Seventeen species and strains of <u>Fusarium</u> were tested with the rays of the Cooper Hewitt Mercury Vapor Lamp. In the following table the host plant is recorded as several of the cultures were unidentified and since the main purpose of the work was that of determining the reaction of the group to the ultra-violet radiations.

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The identified strains will be listed under their species name while the unidentified strains will be listed under the name of the host upon which they occurred.

Table 3. Reaction of various strains of <u>Fusarium</u> to ultra-violet radiations.

Name	3	Sporulation									
		Ultra	-violet :	Darkı	ness						
		Macrop	s.Microsp.:	Macrosp.	Microsp.						
Fusarium	(Aster										
	stalk)	Many	Many	Few	Many						
	(Aster	-	÷		•						
	root)	Many	Few	Many	Few						
	batatig	None	None	None	None						
H-	caliste-										
	phii	Many	Few	Few	Few						
	congluti-										
	nans	Few	None	None	None						
10 -	culmorum	Manv	Many	Few	Few						
*	(Dahlia)	Many	Few	Many	Few						
*	lini	None	None	None	None						
11	lvcoper-										
	sici	None	None	None	None						
*	niveum	None	None	None	None						
H	(Onion)	Few	Menv	None	Few						
	orthocera	9									
		Manv	Many	None	Tew						
*	oxvsporiu	m									
	gladioli	None	None	None	None						
14	radicicol	8	21 0 21 -	1.011.0							
		Manv	Tew	Few	Few						
*	(Pine)	Few	Many	None	Few						
W	trichothe	_ ~ ~	J								
	cioides	Few	Manv	Few	None						
•	an (anil)	Monu	Teur	Tem	Tew						

The cultures in Table 3 were all treated under the same conditions, except for the application of ultraviolet radiation. This exposure was for a period of 30 seconds at a distance of 30 centimeters. All cultures were held at room temperature or slightly above. The chambers where the cultures were held before and after exposure ran at 23 to 24°C. which proved to be the optimum temperature for growth of the majority of the fungi used.

The sporulation is listed in Table 3 under the head of ultra-violet light and darkness. Ordinary daylight illumination was not listed since the reaction in this group is the same in darkness and daylight as shown in Figure 4.

In most all cases the colonies of Fusarium showed a flattening of the mycelial mass in the treated area, sometimes there is a recuperation after treatment and there is merely a flattened surface in the inner part of the colony. In other cultures, of different species, there will be a flattened ring, the colony is fluffy both inside and outside of this ring. The third reaction is a flattening of the entire colony, no signs of fluffiness of growth is ever evident in the treated plates. Figure 5 shows this reaction very clearly, Plate A was treated and it will be noted that it looks entirely barren, yet the entire plate is covered with a thin sheet of mycelium. Plate B is untreated and the fluffiness of the colony is evident. The spore production was not considered since no spores were produced in either of these cultures. Note in Figure 6 that the colony is

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flattened but not made barren. Spore production was affected only slightly.

In comparison note <u>Verticillium glaucum</u> in Fig. 7 when treated with ultra-violet light. Unlike most of the species of <u>Fusarium</u> there is a complete suppression of spore production in this fungus when treated with ultraviolet light. The treated plates were entirely barren.

Figures 8 and 9 show photomicrograph of treated and untreated areas and their respective spore reduction. It will be noted in these plates of <u>Helminthosporium sativum</u> that there is a suppression of spore production within the treated area, as compared to the untreated portion. This genus is similar to <u>Verticillium</u> in this action.

RHIZOCTONIA

The genus <u>Rhizoctonia</u> is of such common occurrence in the soils of Colorado that collection of various strains is extremely easy. Five different cultures of this genus have been isolated and studied. They represent two definite species of <u>Rhizoctonia</u>, <u>R. solani</u> Kuhn. and <u>R. crocorum</u> (Pers.) D.C. The various strains were isolated from the following hosts: <u>Solanum tuberosum</u>, <u>Solanum Jamesii</u> and <u>Beta vulgaris</u>. Four of the cultures were determined as strains of <u>R. solani</u> and one as <u>R</u>. crocorum, the different strains had cultural differences













but their general appearance was almost the same.

Rhizoctonia is similar to most other fungi in that it is very sensitive to temperature changes, for this reason special precautions were taken to keep the temperature constant. Age of colony and the length of time that the fungus had been carried in culture were also found to be very important factors in the response to ultraviolet light.

The usual reaction of <u>Rhizoctonia solani</u> to ultraviolet light is the formation of a sclerotial ring. Fig. 10 shows a photograph of a representative colony after irradiation. A sclerotial ring is formed with a few days after irradiation, making the size of the colony at the time treated. The sclerotial bodies form with age on the check, but not in any ring-like arrangement. In Fig. 11 the effects of ultra-violet light radiations are shown on <u>Rhizoctonia crocorum</u>. A ring due to mycelial knotting is formed, note that no sclerotia are formed in this ring. They may be noted on the outside of the ring beyond the influence of the light.

The following table shows the reaction of the various strains of Rhizoctonia to ultra-violet light.

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Fig. 10. Rhizoctonia solani. A, treated plate, B untreated plate. Note sclerotial ring formed in treated plate, this marks the size of colony at time of treatment.



Fig. 10. Rhizoctonia solani. A, treated plate, B untreated plate. Note sclerotial ring formed in treated plate, this marks the size of colony at time of treatment.





Table 4. Reaction of strains of <u>Rhizoctonia</u> to various kinds of light.

Spe	cies -		Day-		UVL	rayan adar salar na ang ang ang ang ang ang ang ang ang		and a second
strain		Source	light		light	Darkness		
Rhi	zoctonia solani	Solanum tuberosum	scle	r-s	scler-i	scle:	r-va	
R.	solani	Solanum Jamesii		8	* 8	# :	VE	
R.	solani	Solanum tuberosum	×	8	•		a	
R.	crocorum	Betae	. #	VS	* r	#	VB	

s= sclerotial formation is rather slow; i= extremely rapid; r=rapid formation; vs=very slow in formation; a= sclerotial formation absent.

These colonies different normally in cultural characteristics also differed when treated with ultraviolet light. The difference in the reaction of the various strains of <u>Rhizoctonia</u> to ultra-violet light may in time give some rapid method of determining the strain which is present.

There is a tendency for the light to speed up the process of sclerotial production in <u>R</u>. <u>solani</u>, A sclerotial ring appeared five days after treating the culture, while no sclerotia were formed on the check (daylight) until a period of ten days had elapsed. It will be noticed in Fig. 10 that the sclerotia are formed on only the youngest and newest tissue, in the case of the treated plate, while in the check it is the older tissue that produce the sclerotia.

Some species of the genus <u>Selerotium</u> react similarly to R. solani in the production of a sclerotial ring.

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Figure 12 shows <u>Sclerotium intermedia</u> which has been treated and subsequently formed a sclerotial ring. In untreated plate B, it will be noticed that there are no sclerotia formed, while in A they are rather profuse. It can be said that sclerotial production is greatly stimulated in this species when treatments of ultra-violet light are given.

PLEOSPORA HERBARUM

In some of the Ascomycetes studied, the perfect stage was produced when cultures were treated with ultraviolet light. Ordinarily these cultures did not form perithecia and ascospores. The work of Stevens (20) on <u>Glomerella</u> was repeated with the same results as he reports. In addition to the production of the perfect stage on <u>Glomerella cingulata</u> as reported by Stevens the writer has readily produced the ascomycetous stage of <u>Macrosporium parassisicum</u>.

The cultures of <u>Pleospora herbarum</u> used in the above test were secured by treating cultures of <u>M. parasiticum</u> in the open light of a Quartz Mercury Vapor Lamp. The tops were removed from the plates to be treated, and the process carried out as explained under "methods". Plates were divided into three groups; one was treated as shown above, the other was placed in the dark immediately after

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the plates had been inoculated, the third set was run parallel with the two mentioned except that the plates were allowed to be exposed to the ordinary daylight during the entire experiment.

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The following table shows the effects of various kinds of light on the sporulation of <u>Macrosporium parasi-</u> ticum.

Table 5. Reaction of <u>Macrosporium</u> parasiticum to different kinds of light.

	Ring	Perithecia	Conidi a	
Continuous daylight	*	*	X	
Ultra-violet light	X	X	x	
Darkness	-		X	

Single spore isolations were made of <u>Pleospore</u> <u>herbarum</u>, and the resulting growth noted. It was found that the germinating <u>P. herbarum</u> spores gave a mycelium which was typical of <u>Macrosporium parasiticum</u>, and the spores produced were not <u>Pleospora</u> but <u>M. parasiticum</u> instead. Figure 14 shows the <u>M. parasiticum</u> spores obtained from the above mentioned cultures of <u>P. herbarum</u>. This colony, however, when treated with ultra-violet light would again form perithecia and produce the ascospore stage, if not treated it would continue to produce the conidial stage. Figure 13 shows an ascus and ascospores as obtained from treated plates of <u>M. parasiticum</u>, these spores are identical to the exicatti specimens on hand. The type of germination of these spores is shown in Fig. 15











Fig. 15. Photomicrograph of <u>Pleospora</u> <u>herbarum</u> spores showing typical germination.



Spores from exicatti which were collected in 1889 were germinated in like manner as above, they gave the same reaction as the fresh spores which were germinated.

BOTRYOSPHAERIA

One species of Botryosphaeria was placed under test at the beginning of the ultra-violet light work. This species was classified as B. ribes and it had been isolated from apple twigs some eighteen months before the work of the light rays was begun. At no time had this culture produced fruit during the entire cultural period. Ring formations were apparent in this species and it was for this reason that it had been carried thruout the work. Several cultures of B. ribes were treated for periods ranging from 30 seconds to two minutes under the open arc of a Quartz Mercury Vapor Lamp. Within five minutes after treatment small hyaline bodies were found to be forming within the treated plates, at the end of twelve days the bodies took on a brownish-fuscus color and began to assume cuneiform appearance. It was at this period that some of the bodies were crushed and long spores were found within. These spores represent the pycnidial stage of the fungus.

It will be noted that in the above case that there is a stimulation effect in spore production. No spores are formed other than in treated plates.

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PENICILLIUM AND ASPERGILLUS

Species of <u>Penicillium</u> and <u>Aspergillus</u> are affected similarly by radiations from lightof short wave lengths. Both genera show a marked change in sporulation, usually by forming a spore ring.

Mycelial growth in all species studied is very scarce, and is not affected to any appreciable degree by the radiations.

The following table gives the results of <u>Penicillium</u> and Aspergillus as affected by ultra-violet light.

		Spore	Colony	
		ring	flattened	
spergillus	cluvatus	х		
i M	flavus	x	x	
	fumigatus	x	-	
*	glaucus		-	
*	mentii	-	-	
	nig er	x	-	
*1	ochroceus	x	x	
	terrens	x	x	
Ħ	sp.	x	x	
enicillium	communi	x	-	
	citrinum	x	-	
tt:	chrysogenum	x	-	
H er	digitatum	x	-	
	echinatum	x	-	
11	expansum	-	-	
*	gladioli	x	x	
*	humicoli	x	-	
H	purpurogenum	x	-	
ŧ	stoloniferum	x	x	
In the	above listed	species no	change in color wa	8
ver noted i	In the treated	plates.		

Table 6. Effects of ultra-violet light on Aspergillus and Penicillium. Not all species of the two genera act the same toward radiations of light of the short wave lengths, some species form a spore ring within four days after radiation while others will show no ring at the end of a ten dayperiod. The work has been conducted under controlled conditions and no chance for difference in treatment would give an answer to this question. Those species showing no ring formation in irradiated plates were tested at different ages and for varying periods of exposure, yet in no case could a spore ring be formed.

The sporulation is evidently due to some change in the mycelium itself, yet at present it is impossible to say how this change is brought about.

MUCOR AND RHIZOPUS

None of the species treated of these two genera are affected greatly by ultra-violet light. The speed of growth appears to result in negative reaction when an entire colony is considered. There are no appreciable results on the colony as a whole, though if only the end branch of a filament is considered one will notice a killing effect. This effect is first evident due to a stopping of the flow of protoplasm within the plant cell, later there is an excess of branching of the mycelium. These reactions were noted while using a quartz lens to focus the beam of light on the filament as described under methods

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in the forepart of this paper.

It can be stated that in no case where plates were treated for periods short of that causing killing, did a change in sporulation occur. The genera of <u>Mucor</u> and <u>Rhizopus</u> are therefore not affected in so far as spore production is concerned.

The effects of ultra-violet light on growth of <u>Mucors</u> are further considered under, "Effects of Ultra-violet Radiations on Growth".

PHOMA

The genus <u>Phoma</u> in its reaction to ultra-violet light is one of the most interesting groups of fungi studied. Eleven strains of <u>Phoma betae</u> were tested to determine the difference in reaction of strains within a species. All strains were single spore isolations and should represent single spore isolations if such exist.

Those strains which were isolated from sugar beets are given only by number. They are each characterized by their sporulation as well as their cultural characteristics

It was noticed that in some strains pychidia were not produced and the only method of propagation was by means of a transfer of sterile mycelium. This gave a chance to prove whether there could ever be a stimulation due to the radiations of ultra-violet light as shown by the production of spores.

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Striking results were obtained with three of these strains. Treated plates showed small pycnidia formed three days after radiations, while the checks showed no pycnidia at any time.

Those strains which ordinarily fruit in culture were less noticeably affected by the radiations, the only change which could be noticed was that of a grouping of the pycnidia in rings.

In the following table a resume of the tests on the species of <u>Phoma</u> is given.

Table 7. The effect of ultra-violet light on some species (strains) of the genus <u>Phoma</u>.

Specie	ss(stra	ain)	Py	cnidi	2	Colony	Color	
	-					flatten ed	Change	
			D-L	Dark	UVL			
Phoma	betae	(77)	x	x	x	x	X	
Phoma	beta e	(75)	х	x	x	x	-	
Phoma	betae	(122)	x	x	x	X	x	
Phoma	betae	(71)	-	-	-	x	X	
Phoma	be tae	(70)	-		x	x	-	
Phoma	betae	(68)	х	x	x	x	x	
Phoma	be tae l	(76)	-	-	-	х	-	
Phoma	beta e ((80)	х	x	x	x	-	
Phoma	betae	(72	х	x	х	x	x	
Phoma	betae	(148)	-		x	x	x	
Phoma	be tae	(217)	-	-	x	x	x	
Phoma	. sp.(aster							
	roo	ot)	x	х	x	x	-	
Phoma	lingha	am			-	x	-	

The table above shows that not all strains of the same species react the same to ultra-violet light. Some cultures which are apparently sterile have been made to produce pycnidia, while others have not. There is usually a change in the color from whitish to grey blue after treatment, though this does not occur in every strain.

MISCELLANEOUS GENERA

Some genera are not discussed here in detail, due to negative results of their reaction or their similarity to those discussed. In Table 2 the effect of light on any species tested is given. Negative results are shown in the species of <u>Colletotrichum</u>, there is apparently no effectproduced when cultures of this fungue are subjected to ultra-violet light. It was for this reason that some genera were omitted from the detailed discussion.

HEAT AS A POSSIBLE CAUSE FOR CHANGE IN

SPORULATION OF CERTAIN FUNGI.

It was thought for some time that the heat produced by the Quartz Mercury Vapor Lamp at the short range of exposure might be the cause of the peculiar reactions noted in treated cultures of fungi.

Figure 1 shows the comparative rise in temperature as related to the time of exposure. Cultures exposed for only a short period of time (30 seconds) had little chance to be affected by the heat produced, while those exposed for a two-minute period would be heated to a rather high degree. It was from these observations that cultures to be treated for long periods of time were placed on ice cakes while they were being treated. This held the plates

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at 22°C. thruout the exposure.

In order to further test whether or not heat was causing this reaction another type of experiment was conducted, whereby an apparatus was made from an electric toaster so that the wires would be exposed to the surface. Two sets of cultures were then exposed, one to ultraviolet light and the other to heat from the toaster. The two were run simultaneously so there would be no difference in ages of the cultures. The time of exposure to the heat varied from 30 seconds to two minutes in different cultures and at a distance of 14 centimeters, since this distance was found to give the same rise in temperature in a given period as the Mercury Vapor Lamp.

Culture of <u>Rhizoctonia</u> solani showed no signs of a sclerotial ring being formed in the cultures treated with heat, however those treated with ultra-violet light did form a decided ring as shown in Figure 10. Other fungi were treated in similar manner and evidenced no reaction to the heat. It would seem from the above observed facts that the heat produced by the Quartz Mercury Vapor Lamp would be of little weight as affecting sporulation in fungi over the periods of exposure used in above experiments. The stimulative effect is beyond doubt due to rays of short wave lengths.

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EFFECTS OF ULTRA-VIOLET LIGHT ON RATE OF

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GROWTH

The difference in the rate of growth of colonies treated and those which were used as checks has suggested a series of tests dealing strictly with the rate of growth. Three species of <u>Mucor</u> were planted on potatodextrose agar, these were allowed to grow for 24-48 hours at which time exposure and measurements were made. The covers were removed from the plates and the colonies were treated with ultra-violet light as follows: plates were securely placed on the stages of microscopes and ultraviolet light focused on the tip of hypha. A micrometer eyepiece was used to determine the rate of growth of the filament during the time of exposure. The rate of growth was checked every 15 minutes and its value recorded.

Plates from each of the species of Mucor were divided into five groups depending upon the treatment which they were to receive. The first group received moderate exposure (30 seconds), the second group received a long exposure (75 seconds), the third group received 25 seconds treatment but in intermittent exposures of 5 seconds each, beginning at the end of 45 minutes checking. The fourth group was treated similarly to the third group except five, two-second exposures were used at 15 minute intervals. The fifth group was placed under similar conditions -54-

but received no treatment of ultra-violet light.

Plates given different treatment showed a marked difference in their reaction. Figure 16 shows the effects of the various treatments. The curves in this figure are shown as a, b, d, c, and e.

- a = fifth group no irradiation
- b = third group 30 seconds intermittent at
 5 seconds each
- c = first group 30 seconds at one exposure
- d = fourth group 10 seconds intermittent at
 2 seconds each
- e = second group = 75 seconds at one exposure

In curve "a" we find there was no change in the rate of growth when an average of several filaments were considered. Any deviation from this rate of growth is very easy to detect since its plotting represents a straight line.

In curves "b" and "d" one finds an entirely different action than found in the non-treated plates. These plates received intermittent exposures as shown above.

The curves show that the intermittent light will give the same results but in different degrees. The killing effect takes place but its action is much slower than that represented by curve "e" where a single long exposure is made. The curves "b", "d", and "e" show the difference


in long and intermittent exposures.

Curve "c" represents a very peculiar action manifest by <u>Mucors</u> when placed under a moderate exposure for only one treatment. The time here represented is 30 seconds, more than shown in curve "b". It is shown here that there is first a retardation effect, similar to that of treatments of a shorter period, yet after about one and onefourth hours the fungus apparently recovers and the normal rate of growth is again resumed. The final curve will be seen to be approaching that of the check. In this case the filament is not killed but only slowed up in its action.

The killing effect of light of short wave lengths seems to be due to a coagulation of some or all of the protoplasm of the cell. Bovie (3). It has been noticed in the above treatments that there was a coagulation or killing effect taking place within several filaments. First a rapid streaming of protoplasm occurred followed by a swelling just back of the filament tip, no increase in length could be noticed, only an increase in width, this was followed by a stopping of the visible flow of the protoplasm and a bursting of the end of the wall of the filament. The protoplasm would slowly ooze out of the break and coagulate on the outer surface. The possible explanation of this phenomena may be the fact that the more delicate tissue of the filament is near the tip, when the

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ultra-violet light strikes the filament the tip is killed, and since mucors are non-septate there is still a protoplasm pressure coming from the absorption area at the base of this filament, the tip is killed and cannot move, then the sides will swell out and give way to this increase in pressure, only to be ruptured in due time. The above described phenomena takes place more readily under the intermittent treatments. The long exposure seems to retard protoplasmic action within the filament to such a degree that the pressure is not so great as to rupture the filament.

The above mentioned facts lead one to conclude that the tip of the filament is the most vunerable part of the entire plant, since it is the first to be killed when treated. When a killing has taken place in the filament tip there is often a peculiar action manifest in the remaining live filament. Excess branching occurs just back of the dead tip, and is followed in some cases by the formation of a ring by this branching. The appearance of the branching is probably due to back pressure having to have some outlet and the formation of branches is the only means of getting around the dead tip which is blockading the route.

The three species of <u>Mucor</u> tested gave the same results but not identical curves, since some grew more

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readily than others.

From the above results we might say that in no case was a stimulatory effect produced by ultra-violet radiations, regardless of the length of exposure. All filaments were affected by rays, but to different degrees. The results show a retardation or killing effect rather than a stimulation.

There is a great difference in the rate of growth of some organisms even when an entire colony is considered. The foregoing data shows the effect of ultra-violet light on a single filament whereas the following data will show its effect on a colony as a whole.

Figure 17 shows the effects of ultra-violet radiations on the culture as a whole. It will be noted that there is a difference in the size of the two colonies. "A" was treated for the normal period under the ultra-violet light, while "B" received no radiations.

The two colonies show the following relation as to their areas:

At time of
treatmentFour days
after treatmentA - 8.937 sq. cms.10.932 sq. cms.B - 8.670 sq. cms.13.964 sq. cms.

It will be noted that B shows twice the increase in size as compared with A.

The characteristic action of ultra-violet radiations is shown very clearly in A. Here the mycelium is much

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flattened and thickened. The rate of growth is slowed up as is shown by the increase in diameter, yet the colony is not killed or there would be no new growth showing up. This type action is typical of most all fungi which are treated.

The difference in the rate of growth of colonies when treated is usually due to their difference in rate of growth when growing under natural conditions. The slower growing colonies are less affected by these radiations, usually their action is so slight that it cannot be traced. Colletotrichium sp. is a very good illustration of this type of growth. This fungue is apparently unaffected by any radiations which may be given the culture, so long as a drying out of the media is not caused. The rapid growing colonies, Mucor sp., Rhizopus sp., are slightly affected when noted as a whole colony. The action is only on the exposed mycelium and since the growth is so great, then the unexposed mycelium will at once take the place of that which was killed or slowed up. Figure 18 shows the relative penetration of protoplasm by various wave lengths. It will be noted that the longer the wave length the greater the penetration. This slight penetration gives a possible explanation why all filaments are not killed.

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EFFECT OF ULTRA-VIOLET RADIATIONS ON PROTOPLASMIC

MOVEMENTS

The characteristic protoplasmic movement noted in <u>Mucor</u> and <u>Rhizopus</u> is affected greatly by ultra-violet radiations. This streaming is ordinarily found in all the younger filaments of the plants studied, and is continuous throughout the entire plant after a few hour's growth, only to disappear as the plant ages.

The genera of Rhizopus and <u>Mucor</u> are affected similarly by these radiations. The most work reported below has been done with a species of <u>Mucor</u>, although several species of both Mucor and Rhizopus have been tested.

Experiments were conducted with plates containing young cultures, from germinating spores. The plants ranged in age from 24-48 hours. The difference in the age of the colonies was for the purpose of determining the effect of the radiations on protoplasm of different ages. All cultures in a series were of the same age; by starting all cultures from spores it was possible to determine the age of the colony exactly.

Plates were first placed on the stageof a microscope and made solid but adjustable by the use of a mechanical stage, then a filament was placed under the focus of 16 mm. objective. Ultra-violet light was then focused on the filament with a quartz lens and its reaction watched. The time required to cause a ceasation of the protoplasmic flow was found to be dependent on the age of the colony, distance from source of light, as well as the intensity of the light.

It was found that thatportion of the filament nearest the tip and which still shows streaming, is the first to be affected and to cease movement. The time required to cause complete stoppage of flow was found to be directly proportional to the distance from the source of the light.

EFFECT OF ULTRA-VIOLET LIGHT ON METABOLISM

The work of Elfving (13), Bovie (3), Henri (16) and Stevens (20) on the effects of ultra-violet light on organisms was based on the idea that there was some change in the metabolism of the organism due to the action of these rays. In fact it has been stated that the study of the physiology of fungi under the influence of irradiations is one of the most fertile fields of study today.

The respiration of an organism is a possible check which can be used on the effects of such rays on metabolism The CO_2 production was thought to be effected by such radiations. It was chosen as the best measure of respiration under these conditions. An apparatus for this determination is shown in Figure 19. The use of such apparatus gives a very definite index of the amount of CO_2 produced, although it is not a direct quantitative test.

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Carbonic acid is formed when CO comes in contact 2 with the water of a sub-molar base.

 $CO_2 \neq H_2O \implies H_2CO_3$

Carbonic acid is very slightly ionized even when in a dilute condition. It is ionized 0.0017% when in 0.1 molar solution, this slight ionization makes it necessary to have a very weak base in order to show the neutralization effects. A base with a normality of N/18.1 was used to make the determinations.

As near 2 cc. of this N/18.1 base was placed in a container (d) as shown in Figure 19. The exact amount of the base being recorded. The containers were then corked up so that no CO_2 from the air could enter to be taken up by the base.

Figure 19 shows the apparatus used in the determination of the amount of CO_2 given off by the organism during a prolonged period of observation.

In Fig. 19 "a" and "b" represent wash bottles filled with $Ba(OH)_2$ which precipitates the CO_2 of the air by forming $BaCO_3$. The air then passing into "c" is free of CO_2 . "c" contains the organism growing on media to be tested and the container is a quartz tube transparent to the light of short wave lengths. The container "d" has within it a definite amount of a known base. "e" represents an aspirator for the purpose of drawing the air thru

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Fig. 19. Apparatus for determining amount of CO₂ produced by a fungus when under variable light conditions. the circuit and thereby getting all the CO2 formed by the organism.

The action of the apparatus may be described as follows: air is drawn into wash bottles "a" and "b" where it is freed of the CO₂ which it contained. The air is then drawn thru "e" where all the CO2 formed by the fungus is carried on to the solution of NaOH which is contained in "d". The CO₂ here comes in contact with the water of the base and forms H_2CO_3 , which tends to neutralize the NaOH. The amount of neutralization may be definitely indexed by titrating the base which has been treated with CO2 back against a known acid. The actual amount required to neutralize the base will be found to be considerably less in the treated bottles than in those which contain no CO . The exact amount of the acid required to bring about neutrality may be figured from the normality of the acid and base, any difference between the calculated amount and the observed amount will be due entirely to the neutralization effect of H₂CO₃.

In the following table it will be noted that the amount of carbon dioxide produced is very greatly decreased after the fungus has been treated with ultra-violet light. This data indicates that ultra-violet lighthas a retarding effect on the metabolism of a fungus. Respiration is characteristic only of living organisms, and when respira-

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Time	Amt. Na O	Ħ	Norma	lity	Amt.	N/1	NaOH	Normal acid	Calc.	Obser- veđ	NaOH % naatrali- zed
10	2.05cc.	X	.0549	R .	.1125	45cc.		.0288	=3.9100.	3.53cc.	19.02
20	2.12ec.	X	0549	3	.1163	88cc.	. +	.0288	=4.04cc.	3.63cc.	19.33
30	2.00cc. 3	X	.0549	=	.1098	00cc.	. +	.0388	=3.81cc.	3.44ce.	18.50
40	2.10ce. 2	X	.0548	=	.1153	90c c .	. 🔹	•0 288	=4.00cc.	3.60ce.	19.05
50	2.05cc.	X	.05.49	-	.1125	45ce.	•	.0288	=3.91cc.	3.49cc.	20.50
60	2.00cc.	X	•0549	=	.1098	0000.	+	.0288	=3.81cc.	3.42ec.	19.50
	Treated i	for	45 se	econ	ds to	ultr	a-vio	let rad:	Lations -	distanc	e 30 cma.
70	2.00cc. 3	X	.0549	=	.1098	00cc.	+	.0288	=3.81cc.	3.69cc.	6.00
80	2.05cc.]	X	•0549	-	• 112 5	45cc.	+	•0288	=3.91ce.	3.88cc.	1.50
											. *

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tion is lowered there must be either a killing effect of some of the filaments or a reduction to dormancy.

Figure 20. shows the effect of ultra-violet radiations on metabolism of species of <u>Mucor</u>. It will be noted that very little variation is noted in NaOH neutralization until organism is treated, then a great decrease in CO_2 production is noted.

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Fig. 20. Effects of ultraviolet light on the respiration of fungi as shown by NaOH neutralization.

SUMMARY

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A large number of fungi have been tested under the influence of ultra-violet radiations and they have not been found to all give the same reaction.

A wide range of reactions were found to present themselves even within strains of the same species.

A change in the sporulation of an organism is the most common effect that was noted in irradiated plates.

Macrospores are produced in greater proportions in treated plates of Fusarium.

Some species of <u>Rhizoctonia</u> are caused to produce sclerotial rings when treated, others are not.

Sclerotial production is speeded up in irradiated plates of <u>Rhizoctonia</u> species that ordinarily form sclerotia.

The perfect stage of <u>Macrosporium parasiticum</u> formed in plates that were irradiated while the checks did not show the same.

<u>Aspergillus</u> and <u>Penicillium</u> are little affected by ultra-violet radiations, only a formation of a spore ring is evident. Mycelium does not show to be affected.

<u>Mucor</u> and <u>Rhizopus</u> are little affected by ultraviolet radiations when an entire colony is considered.

Ultra-violet light shows a retardation effect on the growth of both <u>Mucor</u> and <u>Rhizopus</u> as noted by single

filament study.

Species of <u>Phoma</u> that normally did not produce pycnidia were caused to fruit when irradiated.

Heat produced from ultra-violet light machine was proven not to be the cause for change in sporulation of fungi.

Vegetative growth is retarded in most all fungi tested.

Ultra-violet radiations will stop the protoplasmic movements in the cells of <u>Mucor</u> and <u>Rhizopus</u>, probably causing a coagulation of the protoplasm.

Metabolic processes are slowed up when fungi are treated with ultra-violet light as indicated by respiration movements.

In none of the experiments here considered did a stimulation effect occur in the vegetative growth.

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