EFFECT OF ULTRAVIOLET AND VISIBLE RADIATION ON THE SPORULATION OF SPECIES OF HELMINTHOSPORIUM

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECT OF ULTRAVIOLET AND VISIBLE RADIATION ON THE SPORULATION OF SPECIES OF HELMINTHOSPORIUM

INTRODUCTION

The genus Helminthosporium includes a large number of species that parasitize bark, leaves and stems of both woody and herbaceous plants. The most widely known of these parasites affect graminaceous hosts. These species cause considerable economic losses to important cereal crops, including barley, corn, rice, oats, wheat and sorghum, in various parts of the world. A large proportion of these species are seed-borne.

Many of the economically important diseases caused by Helminthosporium have been studied by different workers; however, there are very few reports available on the factors that influence reproduction of this pathogen. Among these factors, the most important are light, temperature, hydrogen-ion concentration and nutrition. Excluding the others, light plays an important role in the initiation of the reproductive structures that are so important in identification of species and in the dissemination, survival and perpetuation of the pathogens in nature.

The effects of light on sporulation have been neglected in most studies of diseases caused by Helminthosporium. One of the difficulties in evaluating

or repeating studies which have considered light is that they have not been done under identical conditions, and few have given precise data regarding intensity, duration and quality of light.

The work of Leach (27, p. 66), in which cultures of H. oryzae, H. sativum and H. avenae were exposed to near ultraviolet radiation under reproducible conditions, promoted the idea that it would be interesting to learn what effects visible and ultraviolet radiation had on the sporulation of Helminthosporium species in general. Would there be a common response by members of the genus, or would the requirement of each species be independent of the others? To answer this question, 23 species of Helminthosporium were selected which were mainly parasitic on graminaceous hosts. Most of these species were capable of being disseminated with seed. The study was undertaken with the following objectives:

1. To determine the qualitative and quantitative effects of visible and ultraviolet radiation on different species of Helminthosporium.

2. To determine the effect of ultraviolet radiation on spore morphology of Helminthosporium species.

3. To determine the interaction of ultraviolet radiation and other factors in sporulation and growth of Helminthosporium species.

LITERATURE REVIEW

Introduction

The literature covered in this review is mainly restricted to the effects of ultraviolet and visible radiation on reproductive processes, spore morphology, growth and pigmentation of members of the genus Helminthosporium. Wherever appropriate, references on similar effects on other fungi have also been mentioned. No attempt has been made to review the extensive literature on the germicidal, phototrophic and other miscellaneous effects of light on fungi.

Very few species of Helminthosporium have been irradiated for the purpose of studying the effects of radiation on reproduction and morphology. References on the effects of visible and ultraviolet radiation upon growth and development of other fungi however are numerous, although the lack of adequate information on intensity, duration of exposure and quality of light makes many of these experiments difficult to repeat. In addition, lack of information on, or failure to standardize such important environmental factors as temperature and composition of substrate, makes it difficult to evaluate critically a number of these investigations.

The most recent comprehensive review in the field, is by Bjornsson (4, p. 111). Marsh et al. (32, p. 312) have also compiled a guide to the literature published before 1959. Besides these two sources, excellent general information is available in textbooks on the physiology of fungi by Hawker (16, p. 343; 17, p. 464), Lilly and Barnett (31, p. 464), and Cochrane (6, p. 357-362), and also in the book on the biological effects of radiation by Duggar (13, p. 889-918).

The light which induces reproduction in fungi is mainly confined to the blue or shorter wave lengths, while red or orange have shown little or no effect (18, p. 187). Long exposures to radiation have in general retarded growth (18, p. 187).

For clarification in this review, light responses have been grouped into several categories. Main emphasis has been placed on the effects of ultraviolet light. Visible radiation is considered where it is important for the topic, and in some instances its effect has been compared with that of ultraviolet radiation.

Sporulation: The earliest work on the influence of light and other factors on the growth and fruiting of Helminthosporium sp. was by Johnson (22, p. 797) in 1925. He attempted to find the combination of factors

necessary for inducing sporulation of H. gramineum. Tests were made of the effects of temperature, light, moisture, aeration, hydrogen ion concentration, composition of media, plant tissue extracts, and numerous variations of nutrients on sporulation. In addition, Johnson introduced a number of fungi and bacteria into cultures of H. gramineum in an attempt to induce fruiting. In all his experiments the results were negative. Houston and Oswald (19, p. 1049), however, obtained abundant sporulation in H. gramineum within 48 hours when cultures grown on agar were exposed to solar radiation and subjected to the normal diurnal changes of the environment. They emphasized that light, preferably natural daylight, was necessary for the induction of sporulation.

Although much attention has been centered on the fungicidal action of short-waved ultraviolet radiation, some work has been published on the stimulating action of ultraviolet rays on the reproduction of fungi. Recently Leach (28, p. 156) tested 33 species under continuous near ultraviolet radiation. He concluded that continuous exposure to near ultraviolet radiation induced or increased the sporulation of 31 of 33 species tested. He further observed that whenever irradiation was discontinued, production of fruiting structures

stopped or was greatly reduced. Helminthosporium sativum, on the other hand, produced conidia equally well under irradiation and darkness while H. oryzae formed conidia only when continuous irradiation was followed by a period of darkness of not less than 4½ hours. He further demonstrated that under darkness sporulation of colonies grown on four different media was much reduced in comparison to that of irradiated colonies. Dillon-Weston (12, p. 435) obtained abundant sporulation in Helminthosporium avenae in artifical culture, when colonies were initially irradiated for 10 minutes with a Hanovia quartz mercury vapor lamp, and then irradiated again for 10 minutes six days later. He (12, p. 112) also achieved abundant sporulation in H. avenae and Alternaria solani by exposing them to visible light of high intensity. Johnson and Halpin (23, p. 315) induced cultures of H. sativum to sporulate under illumination but not in darkness.

Stevens (37, p. 210) found that ultraviolet light (Cooper Hewitt quartz mercury arc 21 cm) stimulated pycnidial formation in cultures of Coniothyrium sp.

Lilly and Barnett (31, p. 464) indicated that when cultures of Choanephora cucurbitarum were exposed to alternate light and dark periods of approximately 12

hours each, the average number of conidial heads produced was 1800 to 2000 per culture. Conidia failed to form in cultures exposed to continuous bright light. Christenberry (7, p. 297) found that cultures of Choanephora cucurbitarum did not produce conidia until they had received five hours of darkness alternating with seven hours of light. The greatest number of conidia were produced with 12 hours of light alternating with 12 hours of darkness. Barnett and Lilly (2, p. 84) assumed that two reactions occurred; for the first reaction, light was essential while for the second reaction, a period of darkness was necessary. In continuous light only the first reaction would occur, and therefore, spores would not form. Under continuous light of very low intensity however, both reactions were able to proceed to some extent.

None of the references dealing with the effect of visible and ultraviolet radiation on Helminthosporium mentioned the inhibitory effect of light on growth and sporulation, although inhibitory effects have been noticed in fungi. Dillon-Weston and Halnan (10, p. 962) irradiated Sclerotinia trifoliorum with ultraviolet light from a quartz mercury vapor lamp (12-14 inches distance) and noticed that sclerotia were formed in the medium below the surface. Stevens (36, p. 174) noticed darkening

and distortion or suppression of spore structures in Fusarium batatatis, F. conglutinans, F. niveum, F. vasinfectum and F. spp. when grown on cornmeal agar and exposed for 10, 30 and 50 seconds, to a Cooper-Hewitt quartz mercury arc from a distance of 21 cm. Sporulation of Sclerotinia fructicola was inhibited by light (15, p. 578), while in fungi causing downy mildews of hop, onion, grape, and lettuce (40, p. 365) and Sclerotinia graminicola (11, p. 771) sporulation was more luxuriant in the dark.

In addition to the inhibitory effects of ultraviolet radiation on growth and reproduction, there is also considerable information on the fungicidal effects of ultraviolet radiation. No attempt has been made to review this literature.

Effect of Light on Morphology: Light has been shown to affect the morphology of mycelium, spores and spore bearing receptacles. The only reference to this type of effect in the genus Helminthosporium appears to be that of Houston and Oswald (19, p. 1049). They noticed excessively long conidia of H. gramineum when cultures were subjected to high temperature and light. Similar morphological effects have been reported for other fungi. Leach (27, p. 66) observed that when Ascochyta pisi was irradiated with ultraviolet radiation,

the size of pycnidia decreased significantly with intensity, wave length, and length of exposure, and the shape and size of spores also differed. In Alternaria brassicae var. dauci, Witsch and Wagner (39, p. 310) found a difference in the morphological structures of mycelial strands grown in light from those grown in darkness. The hyphae formed in light were thick-walled, more septate, and had large conidiophores whereas mycelium produced in darkness was less septate, thin-walled and devoid of conidiophores. The greatest spore size in Fusarium coeruleum was obtained when cultures were exposed to light for the first four days after inoculation (35, p. 585). Light from incandescent and fluorescent lamps was reported to induce changes in conidial shape in Alternaria solani, Helminthosporium sativum, Piricularia oryzae, F. moniliforme and Cercospora sp. (24, p. 342; 23, p. 317).

Effect of Visible and Ultraviolet Radiation on Growth: There are only a few reports on the effect of radiation on growth of Helminthosporium. Leach (26, p. 715) found a significant increase in dry weight of shake cultures of H. oryzae grown under continuous near ultraviolet radiation compared with those grown in complete darkness. A stimulatory effect of light on growth of Sclerotinia fructigena was observed (8, p. 131).

Cultures grown in alternating light and darkness were larger than those grown in the dark and those grown in continuous light were still larger. Higher intensities of white light were found to be more favorable for the growth of a Trichoderma sp. than lower intensities, or continuous darkness (25, p. 75). The blue or shorter wave lengths have generally been found to be the most inhibitory for growth, although the growth of several fungi has been stimulated by short exposures to ultra-violet light (25, p. 3). Smoot (34, p. 28) reported that vegetative growth of Diplodia and Phomopsis on agar media was greater in the dark than in cultures irradiated under 80 μw daylight fluorescent lamps or exposed to sunlight. Diener (9, p. 145) observed killing or severe depression of the aerial hyphae of Stemphylium solani when irradiated with a low pressure mercury germicidal lamp (WL-30 lamp, now G30T8) from a distance of 15 cm. He also noticed that the vegetative growth was retarded and that no conidia formed. Colonies were exposed for 30 seconds or more with petri dish covers removed. Johnson (21, p. 227) found that the aerial mycelium of F. batatatis collapsed and there was an increase in pigmentation 24 hours after a 10 minute exposure to Schumann rays (1250 Å-1850 Å). Bailey (1, p. 225) mentioned that direct exposure of

colonies of Fusarium spp. to the mercury arc emitting wave lengths as short as 2230 Å, resulted in a decrease in growth rate. This was also accompanied by stunting as well as injurious effects on mycelium and spores.

Pigment Production: References to pigment production in irradiated Helminthosporium cultures are few. Bean (3, p. 2) noticed a red pigment in colonies of Helminthosporium halodes grown on a special medium in daylight. The pigment was absent when the cultures were grown in darkness. Exposure of H. avenae for 10 minutes each day for six days under a Hanova quartz mercury vapor lamp caused heavy pigmentation and abundant sporulation (11, p. 435), while longer periods of irradiation at higher intensities produced an increase of pigmentation (12, p. 112).

As a general rule, fungi grown in darkness are reported to be less pigmented than fungi grown in normal light (5, p. 373). Duggar indicated that when light promotes pigmentation, the region of spectrum responsible for the pigment production appears to vary amongst different fungi. He also reported that pigment production may be stimulated under blue light and inhibited under red (13, p. 889). McCrea (33, p. 50) associated color production in cultures with the

shorter wave lengths of light, presumably with the ultraviolet light, but irradiation with Cooper Hewitt mercury vapor lamp never gave the same intensity of pigmentation as did sunlight. In general McCrea found that pigmentation in fungi was favored in light rather than in darkness. The effective region was from 3600 Å to 5100 Å. Pigmentation increased slightly from 4000 Å to 4490 Å. with a plateau around 4880 Å. Pigmentation was greatly reduced at 5100 Å and no pigments were produced beyond 5200 Å.

Many pigments have been isolated from fungi (20, p. 526, 678) but correlation has not been found between these and light reactions, indicating the probable complexity of the physiological system responsible for the light reactions in fungi.

MATERIALS AND METHODS

Fungi studied and their origin: Twenty-three species of Helminthosporium were obtained from various sources to investigate effect of ultraviolet and visible radiation on their growth, morphology and reproduction. The following 21 species were obtained from Centraalbureau voor Schimmelcultures Baarn (Netherlands):

1. Helminthosporium gramineum Rabenh., strain Nisikado
2. " oryzae v. Br, de Hann, strain van Raalte.
3. " sacchari Butler, strain Atherton
4. Leptosphaeria salvinii Catt. = Helminthosporium sigmoideum Cav., strain 4
5. Helminthosporium tritici-vulgaris Nisikado, strain NI 4066
6. " victoriae Kingsolver, strain Groves
7. " catenarium Drechsler, strain PD
8. Ophiobolus setariae (Sawada) Ito et Kuribayashi = Helminthosporium setariae Saw., strain 225
9. Helminthosporium sorghicola Lefebvre et Sherwin, strain Lefebvre
10. " turcicum Pass., strain Schwarz
11. " nodulosum (B. et C.) Sacc., strain Nisikade
12. " pedicellatum Henry, strain Machacek
13. " carbonum Ullstrup, strain Gorter
14. " dematioideum Bubak et Wroblewski, strain CBS
15. " alii Camp., strain Grodsinsky
16. " monoceras Drechsler, strain Gilbert
17. " torulosum (Syd.) Ashby, strain CMI
18. " vagans Drechsler, strain Margadant
19. Pyrenophora teres (Sacc.) Drechsler = Helminthosporium teres Sacc., strain Tinline 1
20. Helminthosporium rostratum Drechsler, strain CBS
21. " velutinum Link, strain CBS

H. sativum P. K. & B. was received from Dr. J. E. Machacek, Canada Department of Agriculture, Winnipeg 1, Manitoba and H. siccans Drechsler (Ryegrass isolate) was obtained from Dr. C. M. Leach, Oregon State University, Corvallis, Oregon.

Most of the irradiation work was performed in a controlled temperature room adjustable to any desired temperature from 55° - 100 ° F. The interior of the room was painted black to reduce the reflection of stray light.

Irradiation Chambers: Several types of irradiation chambers were used in this study, all designed by Dr. C. M. Leach (Oregon State University). One chamber was divided into three sections, each with an adjustable shelf which enabled the distance between lamps and cultures to be varied. Each section contained either eight 40 watt daylight fluorescent lamps or eight 40 watt BLB Black Light lamps controlled by time clocks. Lamps were mounted 6½ cm. apart in a holder having a white enamel reflector back and 56 cm. above the cultures unless otherwise noted. To maintain a fairly constant temperature in the chambers, air was kept in circulation by a fan. The recorded variation of temperature at 70° F was ± 0.75° F and at 80° F was ± 1.5° F. In the majority of the experiments a comparison was made between near ultraviolet and

visible radiation. In experiments in which near ultraviolet radiation was filtered from the radiation from daylight fluorescent lamps, a yellow Cinemoid filter (No. 1 Kliegel Brothers, New York) was used (Figure 1).

Another chamber was used specifically for intensity studies. The chamber consisted of five narrow shelves (wide enough for a single row of Petri dishes) at radial distances of 16.8, 31.25, 61.25, 120.0 and 230 cm. from the germicidal lamp. The intensity received from General Electric G30T8 germicidal lamp at the center point of each shelf, as determined from the table presented in Hollaender's "Radiation Biology" (p. 62), ranged from top to bottom as follows: 710, 409, 163, 45 and 13 μ watt/cm^2. The intensity on the top shelf was thus approximately 54 times greater than on the bottom one. A 30 watt, G.E. germicidal lamp (G30T8) was mounted at the top of the cabinet with its longitudinal axis parallel to the shelves. The lamp was connected to a time clock upon which the desired exposure was set. Before exposing cultures, the lamp was allowed to run at least 15 minutes so that the intensity of radiation was fairly constant. Exposure periods were regulated by shielding the lamp with a large plywood shutter mechanism. The full spectrum of

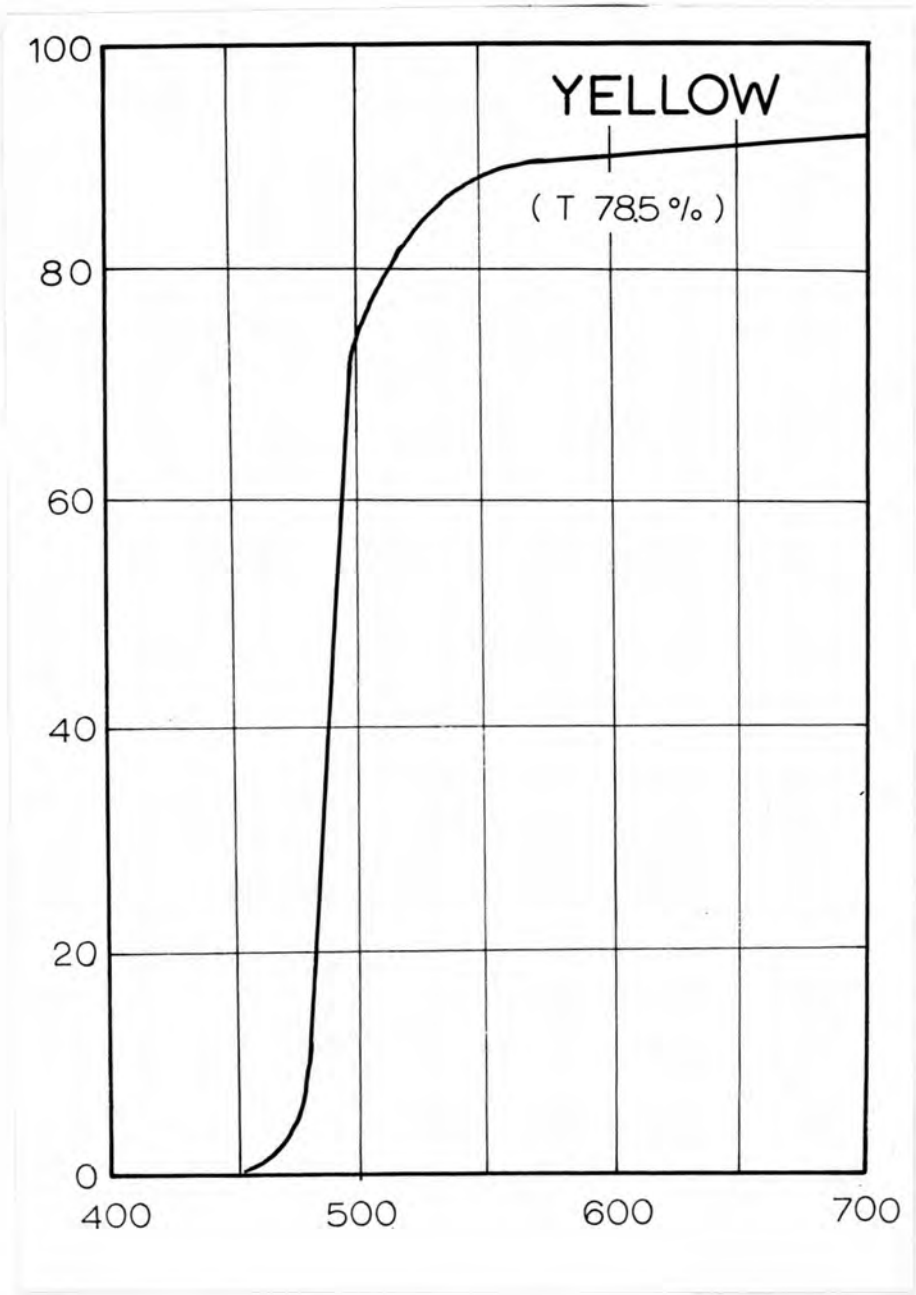

Figure 1. Transmission of the No. 1 Cinemoid (Yellow) filter.

the mercury lamp was employed. Unexposed control cultures were always kept in light-proof containers within the chamber.

A third chamber was used which was particularly suitable for temperature-light studies. Cool white daylight fluorescent type lamps mounted inside the chamber gave intensities ranging from 210 to 500 F. C. in various parts of the chamber. The lamps were connected with a time clock which regulated the light and dark periods. Fluctuation of temperatures was also controlled by clock.

Different Sources of Radiation with Specifications

(1) FLUORESCENT BLACK LIGHT LAMPS: Eight 40 watt General Electric BLB Black Light lamps were used, as a source of near ultraviolet radiation unless otherwise stated. These lamps emit a continuous spectrum between 3200 Å and 4200 Å with a peak intensity of 3650 Å. The spectral curve is given in Figure 2. The intensity of near ultraviolet radiation transmitted through a Pyrex Petri dish lid (average thickness 1.9 mm.) at a 56 mm. distance from eight 40 watt lamps was approximately 120 F. C. using a Weston Model 756 light meter with a quartz filter. As Pyrex Petri dish lids were not removed in the radiation experiments with near ultraviolet, the transmission of pyrex brandglass is shown in Figure 3.

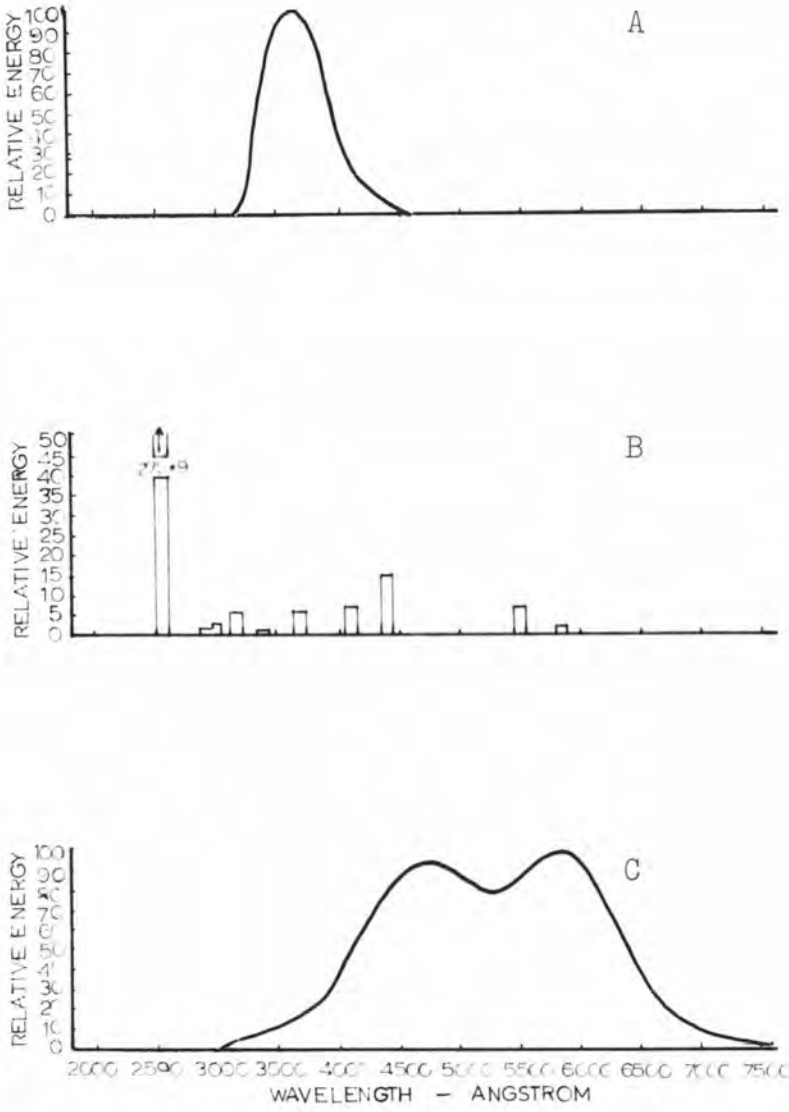

Figure 2. Spectral distribution of various lamps.

A. Fluorescent Black Light lamp.
B. Germicidal lamp.
C. Fluorescent day light lamp.

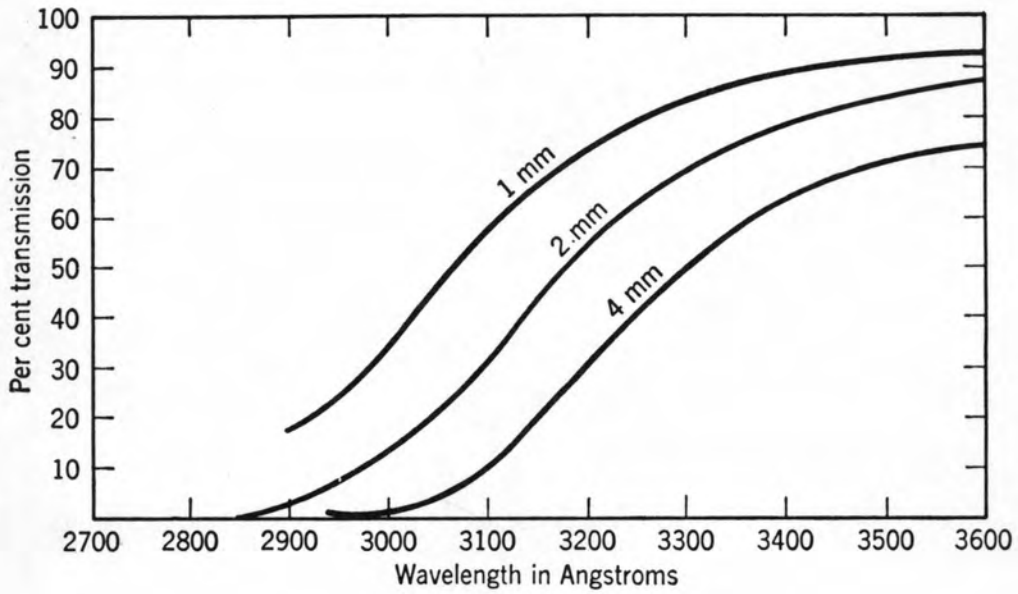

Figure 3. Transmission of various thicknesses of Pyrex clear chemical glass No. 774.

(2) FLUORESCENT DAY LIGHT LAMPS: Eight 40 watt General Electric cool white fluorescent daylight lamps were used for visible radiation. These lamps emit a continuous spectrum between 3666 Å to 7500 Å with maximum emission between approximately 4500 and 6200 Å (Figure 2). Light transmitted through Pyrex Petri dish lids (average thickness 1.9 mm.) at 56 cm. distance from eight 40 watt lamps was approximately 630 F. C.

(3) GERMICIDAL LAMP: G30T8 type General Electric lamp was used as a source of ultraviolet radiation. This lamp emits the typical mercury line spectrum (Figure 2) with approximately 80 per cent of its energy emitted at 2537 Å. The intensity of radiation under this lamp has already been mentioned in the discussion on "intensity chambers."

(4) HANOVIA UTILITY MODEL MERCURY LAMP: This lamp was used as a source of high intensity ultraviolet radiations. It emits a line spectrum between 1849 - 4000 Å, as well as longer wavelengths in the visible spectrum.

Light Measurements and Procedures

(1) MODEL 756 ILLUMINATION METER: Light intensity (foot candles) was measured in many experiments with a Weston Model 756 illumination meter specially fitted

with a quartz filter for measurement of visible and near ultraviolet radiation. The sensitivity of the selenium cell of this meter is much reduced in the near ultraviolet spectrum.

(2) A COMPENSATED THERMOPILE: A compensated thermopile (Kipp and Zonen E20) used in combination with a sensitive galvanometer (Kipp and Zonen A70) was used for absolute measurements of intensity. This thermopile is provided with a fluorite window and with an adjustable slit (10 mm. x 2 mm. maximum opening). Light intensity was measured with the slit at maximum opening and the axis of the slit parallel to the axis of the fluorescent tube. As the thermopile measures only total radiation, it was necessary to determine the percentages of the lamp radiation emitted as ultra= violet and as infra-red energy. Utilizing selective filters (Corning red purple Corex A No. 7-54 and special heat absorbing 1-69), it was found that approximately 22 per cent of the radiant energy from the fluorescent black light lamp was emitted as near ultraviolet.

Cultural Procedures: On the basis of preliminary studies, a 2 per cent potato dextrose agar medium was used unless otherwise mentioned. The unadjusted pH of the medium was approximately 5.8. Twenty ml. of potato

dextrose agar (PDA) per plate was used at all times. Cultures of the same age were in general used to inoculate the plates. Four mm. discs were cut with a cork borer from the periphery of the growing mycelium and transferred to the center of each plate. In experiments with near ultraviolet and visible radiation in which colonies were exposed continuously, or to various cyclical exposures, irradiation was commenced the same day as inoculation. In experiments with far ultraviolet radiation, cultures were allowed to grow for four days at 70° F before being exposed. If the cultures were much older than four days at the time of irradiation, the mycelium of many species tended to completely cover the plates before the end of the tests which hindered the measurement of growth and sporulation. In all the irradiation experiments unexposed controls were held for an equal length of time at the same temperatures as irradiated colonies. After exposure in continuous exposure experiments, cultures were incubated a further 24 hours in darkness at 70° F before being examined. If not examined after 24 hours, they were stored at 5° F under darkness until they could be examined. In most experiments colony characteristics and pigmentation were noted, measurements were made of lineal growth per colony diameters and

number of spores produced per 2 $mm.^2$ area of colony. Microscopic counting of the spores in the colonies was difficult in some species because of the dark pigmentation of the mycelium. Several methods for clearing the colonies were tried; of these a solution of three parts of 95 per cent ethanol and one part of 5.25 per cent sodium hypochlorite ("Clorox") proved most successful. This mixture cleared the colonies and allowed the spores to be observed and counted using transmitted light. Conidia were counted randomly at six places through the colonies. A reticle was mounted in the eye piece to facilitate counting. The total area counted in conidia producing colonies (six places at a magnification of 100X) was 2 mm^2. In colonies with perithecia a total area of 4 $mm.^2$ was counted.

Certain preliminary experiments were performed to show the need or lack of need for certain precautions in procedures. It was found that the age of the stock culture from which inoculations were made had no effect on the results obtained, and for the sake of standardization, 5-10 days old cultures were used for inoculating the plates. During the course of preliminary experiments it was noticed that in cultures of certain species, temperature

exerted a marked effect on the sensitivity of the reproductive processes to ultraviolet radiation, and for this reason standard temperatures were maintained throughout these studies except where interaction of temperature and light was studied. As most of the species grew well at 70° F this temperature was used in most experiments.

As Pyrex brand glass transmits visible radiation efficiently and near ultraviolet radiation fairly efficiently (Figure 3), in all experiments with visible and near ultraviolet radiation the lids of Pyrex Petri dishes were not removed. In experiments with germicidal lamps (mainly far ultraviolet) Petri dish lids were removed prior to exposure and replaced after exposure because Pyrex glass does not transmit far ultraviolet.

EXPERIMENTS AND RESULTS

Effect of Polychromatic Visible and Ultraviolet Radiation on Sporulation

Preliminary experiments were performed in which the 23 species of Helminthosporium (Table 1) were exposed to both visible and ultraviolet radiation and the amount of sporulation determined by microscopic examination. The results of these experiments have been included and described in the appropriate sections. On the basis of these results species were grouped into three provisional categories (Table 1): the first group included species which failed to sporulate in light or in darkness; the second group included those which sporulated equally well in light or in darkness; and the third group included those which sporulated moderately or heavily when exposed to various periods of light and dark, but sparsely or not at all in darkness.

In these preliminary experiments H. tritici-vulgaris, H. pedicellatum, H. gramineum, H. sigmoideum, H. velutinum and H. rostratum did not sporulate when exposed continuously either to near ultraviolet radiation (eight 40 watt BLB Black Light lamps at 56 cm., 120 F.C.) or to visible radiation (eight 40 watt cool white fluorescent lamps at 56 cm., 630 F.C.). In addition, exposures of 2 - 5 minutes to a germicidal lamp (G30T8) at intensities

Table 1. TENTATIVE GROUPING OF SPECIES ON THE BASIS OF THEIR RESPONSE TO IRRADIATION IN PRELIMINARY EXPERIMENTS WITH VISIBLE AND NEAR ULTRAVIOLET LIGHT.

No Sporulation Under Light or Dark	Sporulation Under Light or Dark Similar	Sporulation Light Stimulated
H. tritici-vulgaris	H. nodulosum	H. sacchari
H. gramineum	H. monoceras	H. vagans
H. velutinum	H. catenarium	H. torulosum
H. sigmoideum	H. sativum	H. siccans
H. rostratum		H. alii
H. setariae		H. carbonum
H. pedicellatum		H. turcicum
		H. dematioideum
		H. oryzae
		H. sorghicola
		H. victoriae
		P. teres

ranging from 710 - 13 μ w/cm^2 failed to induce sporulation. Different media (malt, Czapek, oat, corn and PDA) and temperature (41° F - 85° F) were also tried without any positive results.

The ensuing more detailed studies were in general conducted with six species of Helminthosporium which had shown fairly clear-cut responses in the preliminary experiments. Among these species H. setariae did not sporulate in light or in darkness, H. siccans sporulated in light only, H. dematioideum, H. sorghicola, H. victoriae, and P. teres sporulated poorly in dark but moderately to heavily in light.

(1) EFFECT OF CONTINUOUS EXPOSURE: Freshly inoculated plates of seven species of Helminthsporium (Figure 4) were placed under continuous exposure to near ultraviolet radiation (eight 40 watt BLB Black Light lamp at 56 cm.) and under continuous exposure of visible radiation (eight 40 watt cool white fluorescent lamps at 56 cm.) without ultraviolet (cinemoid No. 1 yellow filter). After seven days the cultures were removed from under the lamps, incubated a further 24 hours in darkness and examined for the presence of conidia and for perithecia in the case of Pyrenophora teres. With one exception sporulation in colonies exposed continuously to near ultraviolet radiation was

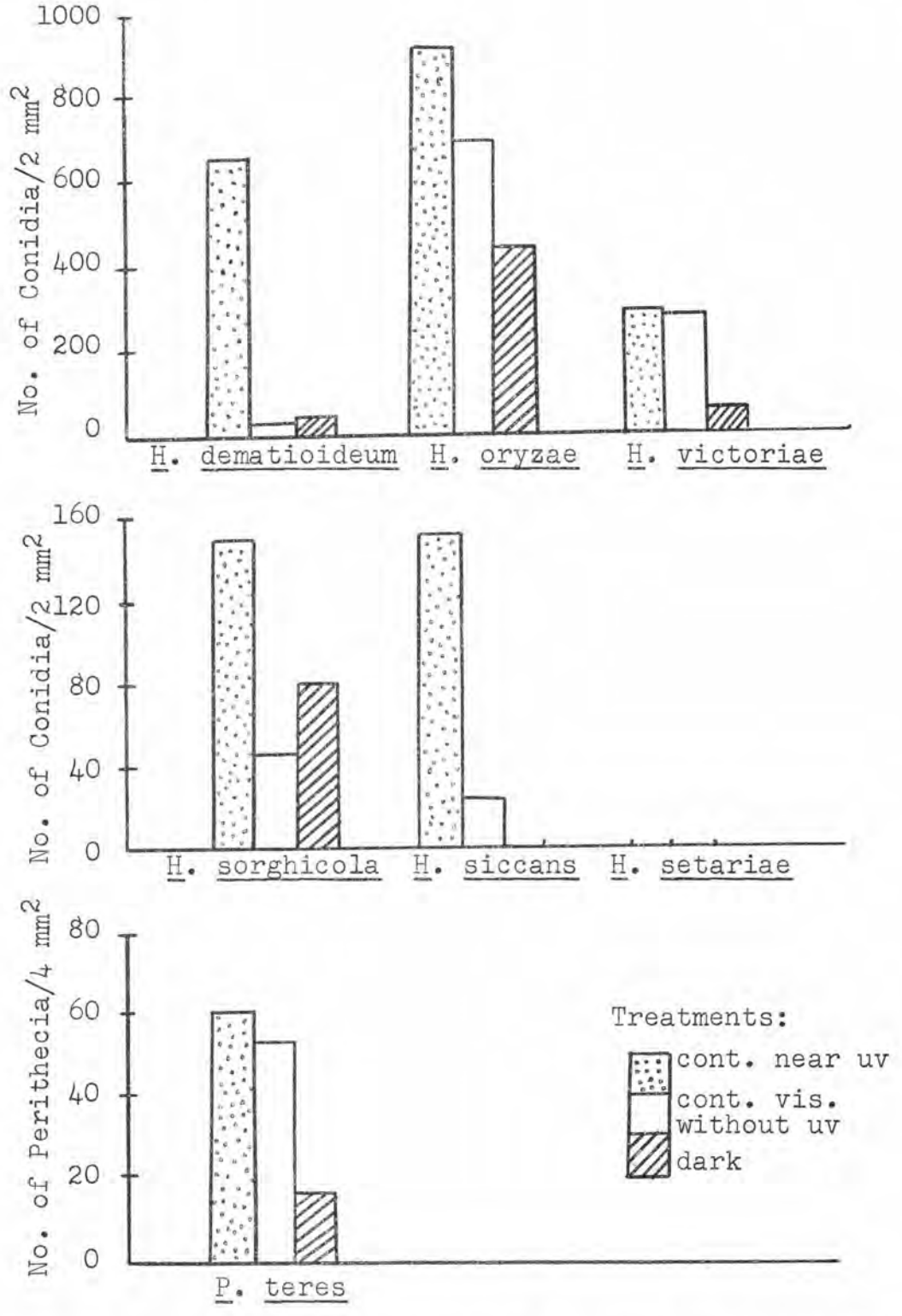

Figure 4. Effect of continuous exposure to visible and near ultraviolet radiation on the sporulation of seven species of Helminthosporium.

greater than for cultures irradiated under visible radiation without ultraviolet, and in darkness (Figure 4). H. dematioideum under continuous visible radiation without ultraviolet sporulated sparsely and the abundance of conidia was similar to colonies grown in darkness. Under exposure to near ultraviolet radiation sporulation was consistently much more profuse. H. oryzae, H. sorghicola, H. victoriae, and Pyrenophora teres sporulated under all three treatments but responses to irradiation varied somewhat between species. Maximum conidial formation occurred under near ultraviolet, with much less sporulation under visible radiation and in darkness. H. victoriae was an exception in which sporulation was equally abundant under visible and near ultraviolet radiation. H. siccans did not sporulate in darkness (Figure 4) while under continuous near ultraviolet radiation, sporulation was highest of the three treatments. H. setariae did not sporulate in any of the treatments and it was concluded that at the intensities used neither ultraviolet nor visible radiation stimulated sporulation. It was also concluded that with the exception of H. setariae, reproduction was stimulated by radiation, particularly near ultraviolet radiation, in all the species tested. As sporulation was consistently greater

in colonies exposed to near ultraviolet radiation (H. victoriae was an exception), it appeared that the wave lengths of electromagnetic radiation most efficient in inducing sporulation were in the near ultraviolet region of the spectrum. There were indications, however, of some stimulation by visible radiation, though this was equal to ultraviolet radiation only in the case of H. victoriae. Because no attempts were made to equalize the intensity of visible and ultraviolet radiation in these experiments, the resulting variation in sporulation may have arisen from quantitative rather than qualitative differences in radiation.

(2) EFFECT OF DIFFERENT LENGTH OF EXPOSURE ON SPORULATION: Three species of Helminthosporium (Table 2) were subjected to various treatments to compare the effect of continuous exposure versus cyclical exposure to both near ultraviolet radiation (Fluorescent Black Light lamp at 56 cm., 120 F.C.) and visible radiation minus near ultraviolet (Fluorescent day light lamp 56 cm., 630 F.C. with a cinemoid No. 1, yellow filter). Freshly inoculated PDA plates were placed under a lamp and irradiated, while unexposed controls were kept in light proof metal containers in the same chamber. After seven days of irradiation some

Table 2. EFFECT OF EXPOSURES TO VISIBLE AND ULTRAVIOLET RADIATION ON THE SPORULATION OF THREE SPECIES OF HELMINTHOSPORIUM.

Species	Number of Spores Per 2 mm^2 (mean) 1/ 2/			
	Continuous Near Ultraviolet	12 Hours Near Ultraviolet 12 Hours Dark	Continuous Visible without Ultraviolet	Dark
H. dematioideum	512	630	14	0
H. victoriae	480	603	409	21
Pyrenophora teres	70	98	64	16

1/ In Pyrenophora teres the number of perithecia per 4 mm^2 were counted.

2/ Seven day old colonies.

of the colonies were examined and the rest were stored in a cold room at 5° F till they were examined. The amount of sporulation was determined microscopically at 100X. The results are consolidated in Table 2.

All three species of Helminthosporium (Table 2) showed greater sporulation in irradiated colonies than in colonies grown in darkness. Most profuse sporulation resulted when periods of irradiation were interspersed with dark periods. H. dematioideum sporulated sparsely under continuous visible radiation without ultraviolet while under continuous near-violet radiation and under cyclical exposure to near ultraviolet and darkness, heavy sporulation was obtained. In colonies of H. victoriae and Pyrenophora teres, continuous exposure to visible radiation without ultraviolet and to near ultraviolet radiation was equally effective in causing sporulation. All three species formed concentric rings of growth under cyclical exposures. In addition to sporulation, several other effects of irradiating colonies were observed. Heavy pigmentation occurred in colonies of H. victoriae grown under continuous near ultraviolet radiation. In Pyrenophora teres, perithecia appeared to mature earlier in cultures exposed continuously to near ultraviolet radiation or to cyclical exposures.

(3) EFFECT OF DIFFERENT EXPOSURES UNDER A GERMICIDAL LAMP: Four species of Helminthosporium were irradiated under a germicidal lamp for different lengths of time (Table 3) in order to determine the effect of sporulation. As the germicidal lamp emits approximately 80 per cent of its radiation at 2537 Å, it was assumed that most of the effects observed could be attributed to far ultraviolet. PDA plates were inoculated with 4 mm. discs and were incubated at 70° F in dark. After four days they were removed and placed in the "intensity chamber" at a distance of 61 cm. from the lamp (163 $\mu w/cm^2$) and lids of the Petri dishes were removed. Exposures ranging from five minutes to one hour (Table 3) were given each day to all four species (Table 3). After each exposure the lids were replaced and cultures were incubated in darkness at 70° F until the next exposure. Colonies were exposed once each day for three days. Controls were kept in light proof containers at 70° F also in the "intensity chamber." Colonies were left for additional 24 hours at 70° F following the last exposure and microscopic observations were then made at a magnification of 100X. The data are consolidated in Table 3.

In H. dematioideum a one hour exposure caused reduction of sporulation. An exposure of five minutes

Table 3. EFFECT OF DIFFERENT EXPOSURES[1] UNDER A GERMICIDAL LAMP ON THE SPORULATION OF FOUR SPECIES OF HELMINTHOSPORIUM.

Species	Number of Conidia[2] per 2 mm² (mean)				
	1 hour repeated for 3 days	30 minutes repeated for 3 days	15 minutes repeated for 3 days	5 minutes repeated for 3 days	Dark
H. dematioideum	141	200	194	475	0
H. setariae	0	0	0	17	0
H. siccans	0	0	0	75	0
Pyrenophora teres	84	107	34	95	13

1/ Exposure at 163 μ watt/cm².

2/ In Pyrenophora teres, the number of perithecia per 4 mm² were counted.

induced maximum conidial production while long exposures were apparently inhibitory. All exposures increased perithecial production in cultures of Pyrenophora teres. Only 15 minutes exposure influenced reproduction while in other there was little difference in the amount of sporulation at the maximum and minimum exposures. The slight suppression of perithecial production of Pyrenophora teres under 1 hour exposure may be due to the ability of the fungus to form perithecia below the surface of the medium. Thus when perithecia are inhibited at a certain intensity at the surface of the medium they are still able to form below the surface at a depth at which the intensity has been reduced below the inhibitory level by absorption of the medium (29). Thus the abundance of perithecial production could be expected to be similar over a quite broad range of exposures though the location of perithecial production relative to the surface of the medium would be quite different.

Diameters of irradiated and nonirradiated colonies (Table 4) were also measured. The growth in all irradiated colonies was less than in colonies grown in darkness, indicating that radiation from the germicidal lamp suppresses growth (Figures 5 and 6). In general growth of exposed colonies decreased as the length of

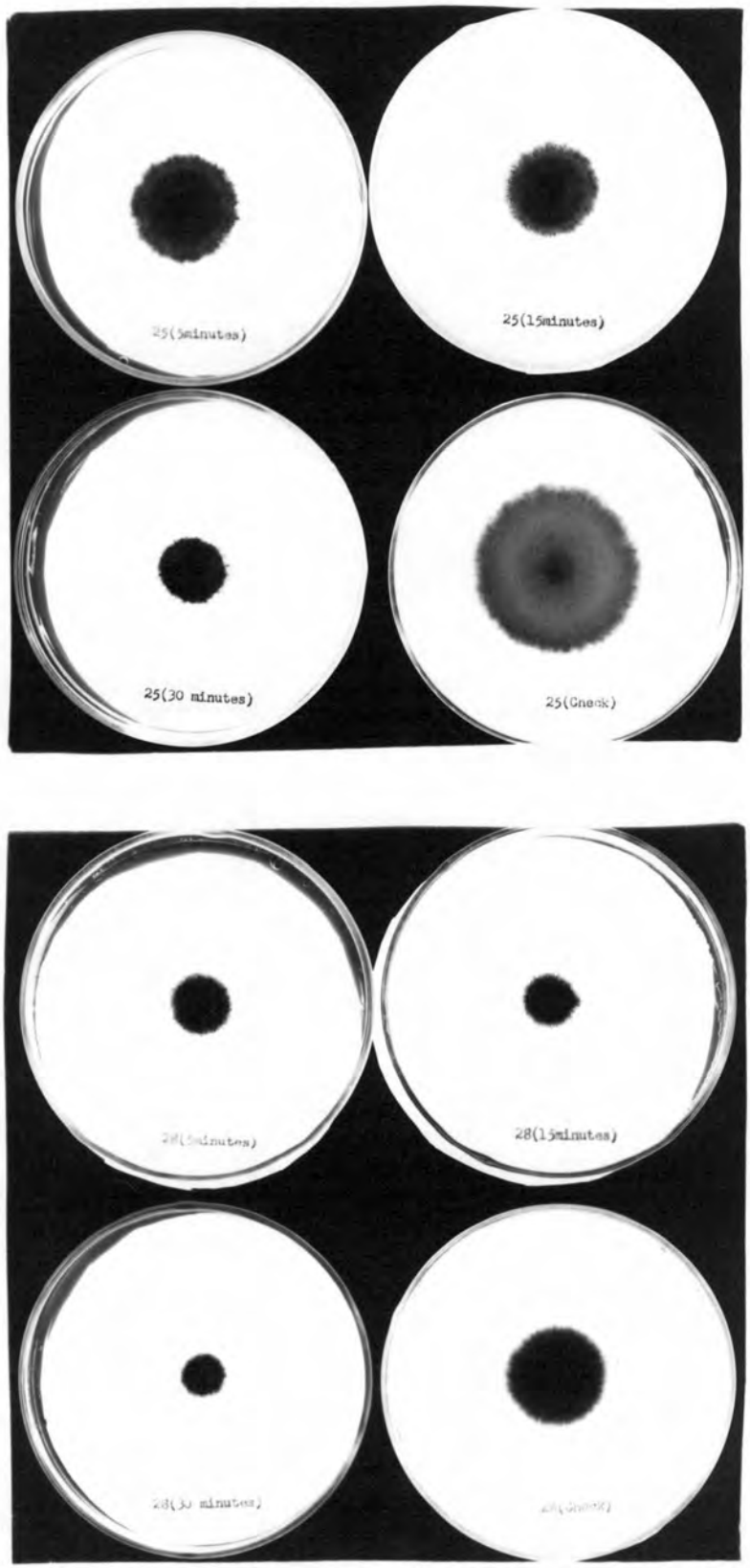

Figure 5. Effect of length of exposure to a germicidal lamp on the growth of H. dematioideum (25) and Pyrenophora teres (28).

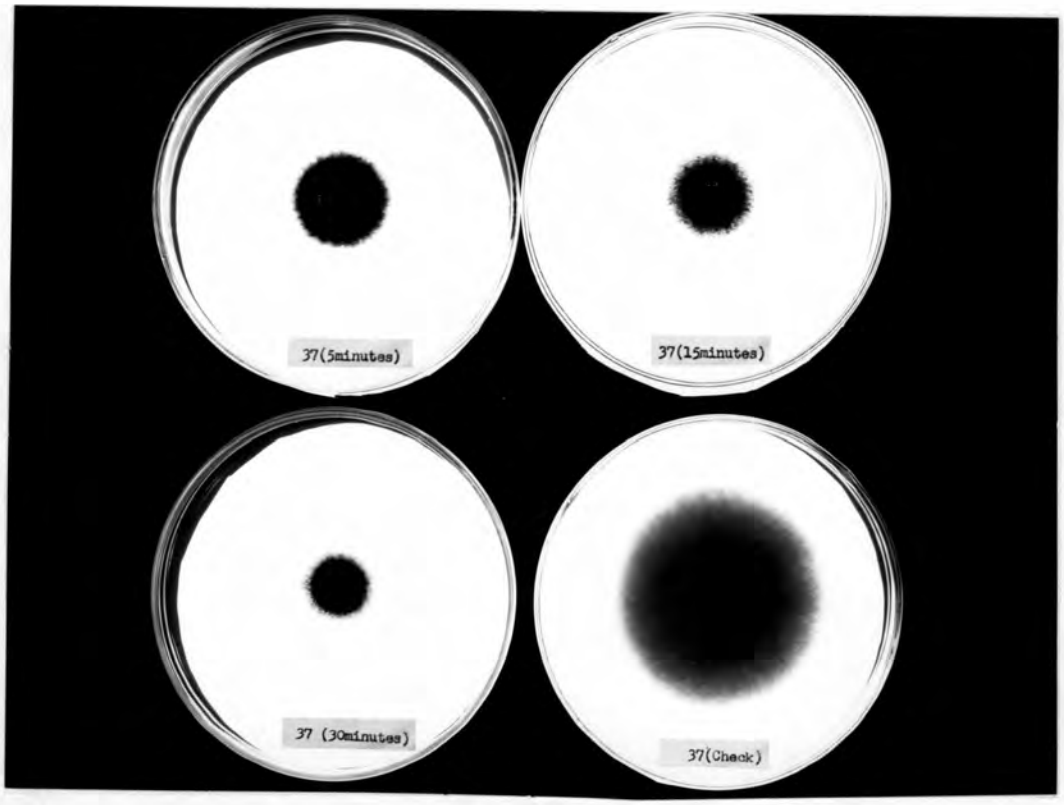

Figure 6. Effect of length of exposure to a germicidal lamp on the growth of Helminthosporium setariae.

exposure was increased. The mechanism of growth and sporulation appeared to be independent of each other. It appeared, however, that inhibition in growth by irradiation caused induction of sporulation or increased the formation of reproductive structures, but as the intensity increased growth and sporulation decreased.

Table 4. EFFECT OF LENGTH OF EXPOSURE[1/] TO A GERMICIDAL LAMP ON LINEAL GROWTH OF FOUR SPECIES OF HELMINTHOSPORIUM.

Species	Diameter of Colonies in mm (mean) [2/]				
	1 Hour	30 Minutes	15 Minutes	5 Minutes	Dark
H. dematioideum	42	44	49	48	58
H. setariae	34	36	36	46	69
H. siccans	30	31	32	33	55
Pyrenophora teres	28	32	34	36	38

1/ Indicated exposures were given once on each of three consecutive days.

2/ Seven day old colonies.

Intensity Experiment with Germicidal Lamp

Experiment No. 1: In further experiments with a germicidal lamp, the effect of intensity of radiation on sporulation was determined for H. setariae and H. dematioideum. The former species up to this time had been a notoriously poor sporulator under irradiation, whereas the latter had sporulated profusely. Both

species were transferred to PDA and incubated at 70° F in darkness. When four days old, the colonies were placed in the "intensity chamber" and irradiated at intensities of 710 μ watt/cm.^{2}, 409 μ watt/cm.^{2}, 163 μ watt/cm.^{2}, 45 μ watt/cm.^{2} and 13 μ watt/cm.^{2}. Each treatment was replicated three times. Lids of the Petri dishes were removed during irradiation. Unexposed controls were placed in a light proof containers in the "intensity chamber" until the exposures were completed. Exposed and unexposed plates were then returned to the 70° F incubator for an additional 24 hours before observations were made.

H. dematioideum sporulated heavily (when exposed for 3 hours) at the lowest intensity of 13 μ watt/cm.^{2} (Figure 7). At higher intensities there was a progressive reduction of sporulation. A shorter exposure of one hour caused the reverse effect although sporulation was only slightly greater at 710 μ watt/cm.^{2} than at the 13 μ watt/cm^2.

H. setariae failed to sporulate at all intensities when exposed for one hour, but sporulated sparsely when exposed at the lower intensities for three hours. Consecutive three hour exposures at the higher intensities inhibited sporulation.

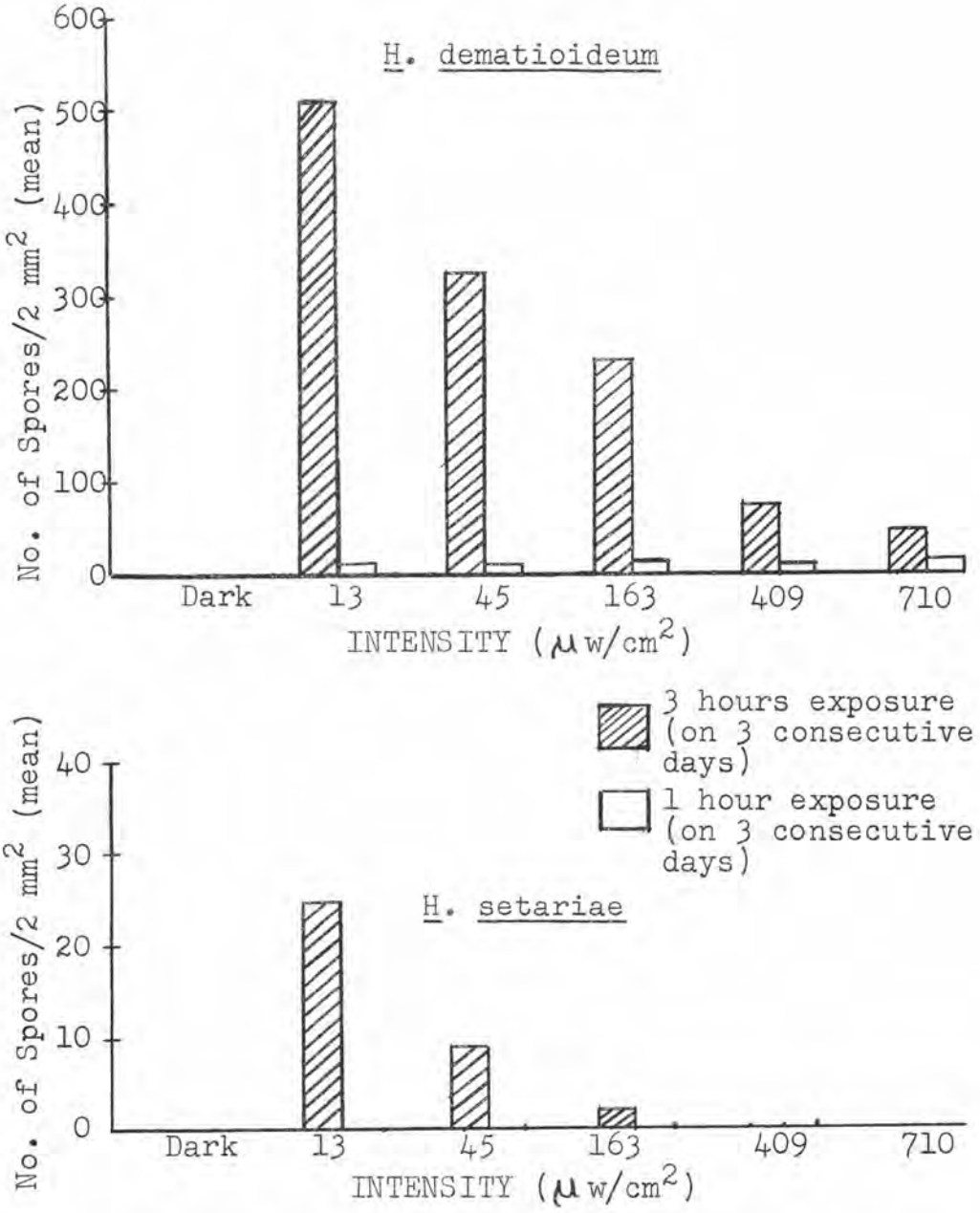

Figure 7. Effect of intensity of radiation under a germicidal lamp on the sporulation of two species of Helminthosporium.

Experiment No. II: Five species (Table 5) were exposed to a germicidal lamp at intensities of 710, 409, 163, 45 and 13 μ watt/cm^2. A single exposure of two minutes was given on each of seven consecutive days. PDA plates inoculated with 4 mm. discs taken from the periphery of dark-grown colonies were incubated for four days at 70° F in darkness. They were then irradiated in the "intensity chamber" with the Petri dish lids removed. Each treatment was replicated three times. Unexposed control cultures were kept in light proof containers in the "intensity chamber." Twenty-four hours following the last exposure the amount of sporulation was rated arbitrarily as indicated in Table 5. Measurements of colony diameters were also made.

H. pedicellatum and H. setariae did not sporulate irrespective of treatment (Table 5). H. sacchari sporulated under both irradiation and darkness, however, profuseness of sporulation was greater under irradiation and increased with intensity. H. siccans sporulated only under irradiation, but sporulation decreased with an increase of intensity. H. torulosum sporulated under both irradiation and darkness, although greatest sporulation occurred at the lowest intensity.

Table 5. EFFECT OF DIFFERENT INTENSITIES[1] OF RADIATION ON THE SPORULATION OF FIVE SPECIES OF *HELMINTHOSPORIUM* EXPOSED TO A GERMICIDAL LAMP.

Species	Amount of Sporulation[2]					
	710 μ w/cm.^{2}	409 μ w/cm.^{2}	163 μ w/cm.^{2}	45 μ w/cm.^{2}	13 μ w/cm.^{2}	Dark
H. sacchari	++++	++++	++++	+++	+++	++
H. siccans	++	++	++	+++	+++	o
H. torulosum	++	++	++	++	+++	+
H. pedicellatum	o	o	o	o	o	o
H. setariae	o	o	o	o	o	o

1/ Exposed two minutes each day for seven consecutive days.

2/ o = No sporulation, + = scarce, ++ = slight, +++ = moderate, ++++ = heavy.

General growth responses of all the species to irradiation at different intensities were alike (Table 6). In most of the species tested (Table 6), growth of irradiated colonies at lowest intensity (13 μ w/cm^2.) was similar to that of colonies grown under darkness. This indicates that radiation at the lowest intensity did not effect growth. Growth under highest intensity (710 μ w/cm^2.), however, was much reduced in comparison to the growth of colonies grown under darkness. It is also apparent from data presented in Table 6 that with the exception of H. setariae, growth decreased as the intensity was increased.

Experiment No. III: Eleven species of Helminthosporium (Table 7) were exposed to Hanovia utility model mercury lamp (emits radiation from 1849 - 4000 Å), from a distance of 25 cm. Exposures ranged from five seconds to one minute (Table 7) and were given on each of three consecutive days. PDA plates inoculated with 4 mm. discs obtained from the periphery of dark grown colonies were incubated for four days at 70° F in darkness. The lamp was turned on for 15 minutes prior to use to obtain constant intensity and colonies were irradiated with the Petri dish lids removed. Each treatment was replicated three times. Unexposed control cultures were kept in light proof

Table 6. EFFECT OF DIFFERENT INTENSITIES OF RADIATION ON THE GROWTH OF FIVE SPECIES OF HELMINTHOSPORIUM EXPOSED TO A GERMICIDAL LAMP.

Species	Growth in mm.					
	710 μ w/cm^2	409 μ w/cm^2	163 μ w/cm^2	45 μ w/cm^2	13 μ w/cm^2	Dark
H. sacchari	29	44	62	69	75	80
H. siccans	70	85	86	90	90	90
H. torulosum	50	55	69	70	78	85
H. pedicellatum	51	61	79	86	90	90
H. setariae	71	85	90	90	88	90

1/ Diameter of 11 day old colonies (mean of three replications)

Table 7. EFFECT OF LENGTH OF EXPOSURE (HANOVIA UTILITY MODEL MERCURY LAMP) ON SPORULATION OF SPECIES OF *HELMINTHOSPORIUM*.

Species	Sporulation[1/ 2/]					
	5 seconds	10 seconds	15 seconds	30 seconds	1 minute	Dark
H. nodulosum	++++	++++	++++	++++	++++	++++
H. monoceras	++++	++++	++++	++++	+++	++++
H. catenarium	++++	++++	++++	++++	++++	++++
H. sativum	+++	+++	+++	+++	+++	+++
H. vagans	+++	+++	+++	+++	+++	++
H. alii	++++	++++	++++	++++	++++	+++
H. sacchari	+	++	++	++	o	++
H. torulosum	+	+	+	++	o	+
H. siccans	+	+	+++	+++	++	o
H. turcicum	++	++++	++++	+++	++	+++
H. carbonum	++	+++	+++	++++	++++	++

1/ o = No sporulation, + = scarce, ++ = slight, +++ = moderate, ++++ = heavy.

2/ Ten day old colonies.

containers at the same temperature. Twenty-four hours after the last exposure, the amount of sporulation was rated arbitrarily as indicated in Table 7.

H. nodulosum, H. monoceras, H. catenarium and H. sativum sporulated equally well in darkness and under the different exposures indicating that under these conditions light is not necessary for the initiation of reproduction. H. vagans and H. alii sporulated both under irradiation and in darkness, but sporulation was greater in irradiated cultures. H. sacchari, H. torulosum, H. siccans, H. turcicum and H. carbonum sporulated more heavily when irradiated than in darkness. The optimum dosage for maximum conidial production in these latter five species varied from species to species.

Effect of Different Interacting Factors and Ultraviolet Radiation on Sporulation

MEDIA AND ULTRAVIOLET RADIATION: To ascertain the effect of media on the sporulation of irradiated colonies, three species of Helminthosporium were grown and irradiated on five different media (Table 8). Freshly inoculated plates were placed under continuous exposure to near ultraviolet radiation (eight 40 watt BLB Black Light lamps at 56 cm.). Controls were kept under darkness in light proof containers. Treatments were replicated three times. After seven days of

Table 8. EFFECT OF MEDIA AND NEAR ULTRAVIOLET RADIATION ON SPORULATION OF THREE SPECIES OF HELMINTHOSPORIUM.

Species	Exposure	Number of Spores Per 2 mm^2 (mean) 1/ 2/				
		PDA	Malt Agar	Czapek Agar	V-8 Juice Agar	Nutrient Agar
H. dematioideum	continuous near ultraviolet	736	360	789	900	442
	dark	32	170	508	751	382
H. victoriae	continuous near ultraviolet	487	222	298	346	316
	dark	17	0	0	2	1
Pyrenophora teres	continuous near ultraviolet	78	62	62	96	80
	dark	14	21	7	32	55

1/ In Pyrenophora teres the number of perithecia per 4 mm^2 were counted.

2/ Eight day old colonies.

continuous exposure, irradiation was stopped and the colonies were incubated a further 24 hours in darkness at 70° F before spore counts were made.

From Table 8 it is apparent that on all five media greater sporulation was obtained in irradiated colonies. The effect of media on reproduction was most pronounced in colonies of H. dematioideum whether grown under radiation or in darkness. H. victoriae did not sporulate under darkness in any of the five media tested except PDA, where sporulation was very sparse. The variation in the amount of sporulation in colonies of H. victoriae on the different media indicated that nutrition influenced the sporulation of irradiated colonies. Perithecia were produced in much greater abundance in irradiated colonies of Pyrenophora teres on all media. In general, the effect of nutrition on reproduction was most pronounced in colonies grown in darkness. Though there were also differences under irradiation, these were not so pronounced. Different media also influenced the growth of all three species tested (Figures 8, 9, and 10). Growth responses of irradiated and non-irradiated colonies on different media varied according to the species. In P. teres perithecia of irradiated colonies matured earlier than the colonies grown under dark except on nutrient agar (Figure 10).

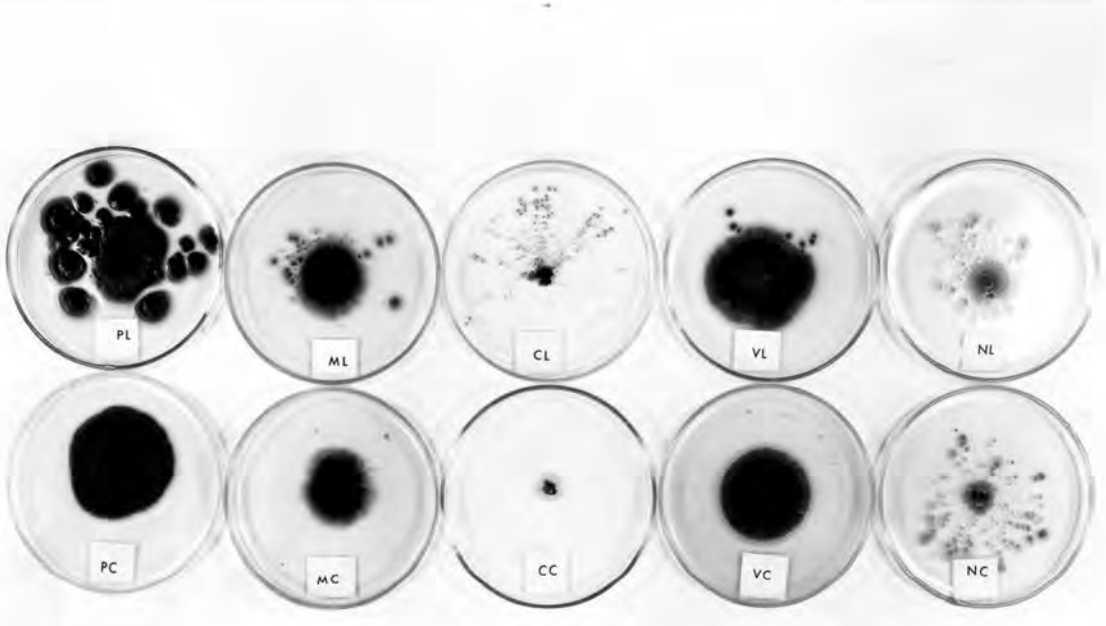

Figure 8. Effect of different media and near ultraviolet radiation on the growth of Pyrenophora teres.

P, Potato dextrose agar; M, Malt agar; C, Czapek agar; V, V-8 juice agar; N, nutrient agar; L, in first row indicates irradiated cultures; C, in second row non-irradiated cultures.

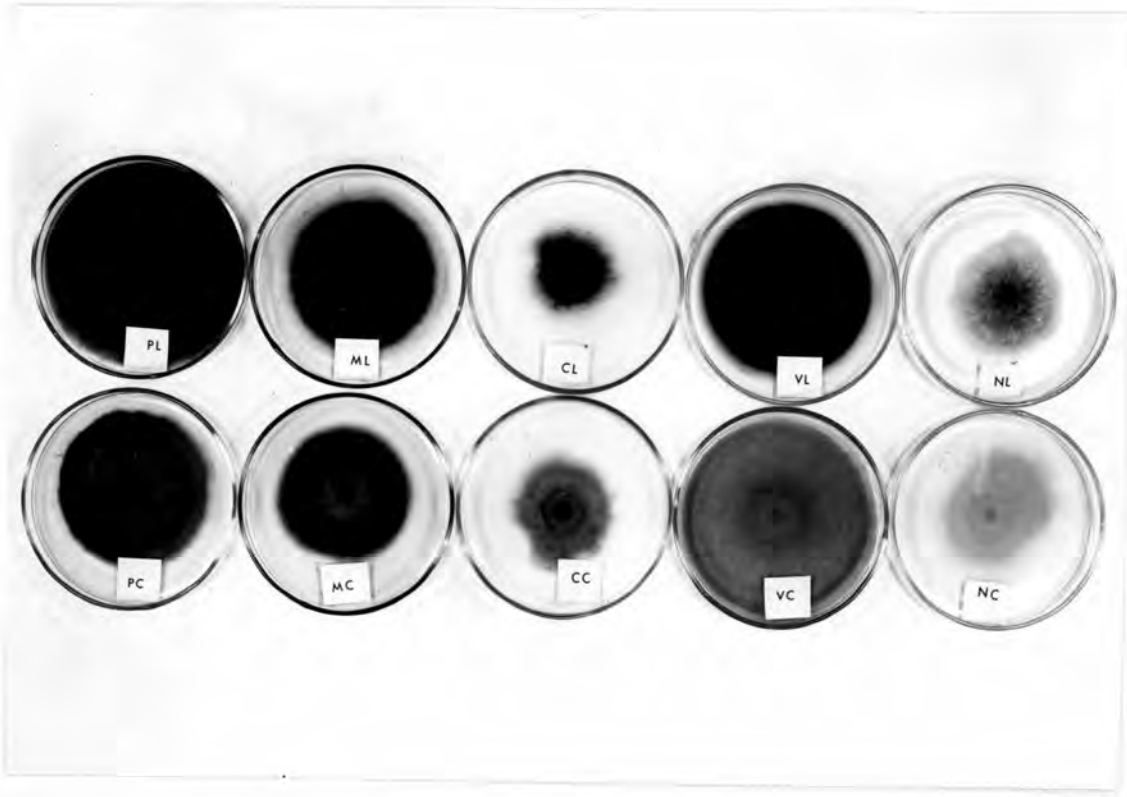

Figure 9. Effect of different media and near ultraviolet radiation on the growth of Helminthosporium victoriae.

P, Potato dextrose agar; M, Malt agar; C, Czapek agar; V, V-8 juice agar; N, nutrient agar; L, in first row indicates irradiated cultures; C, in second row non-irradiated cultures.

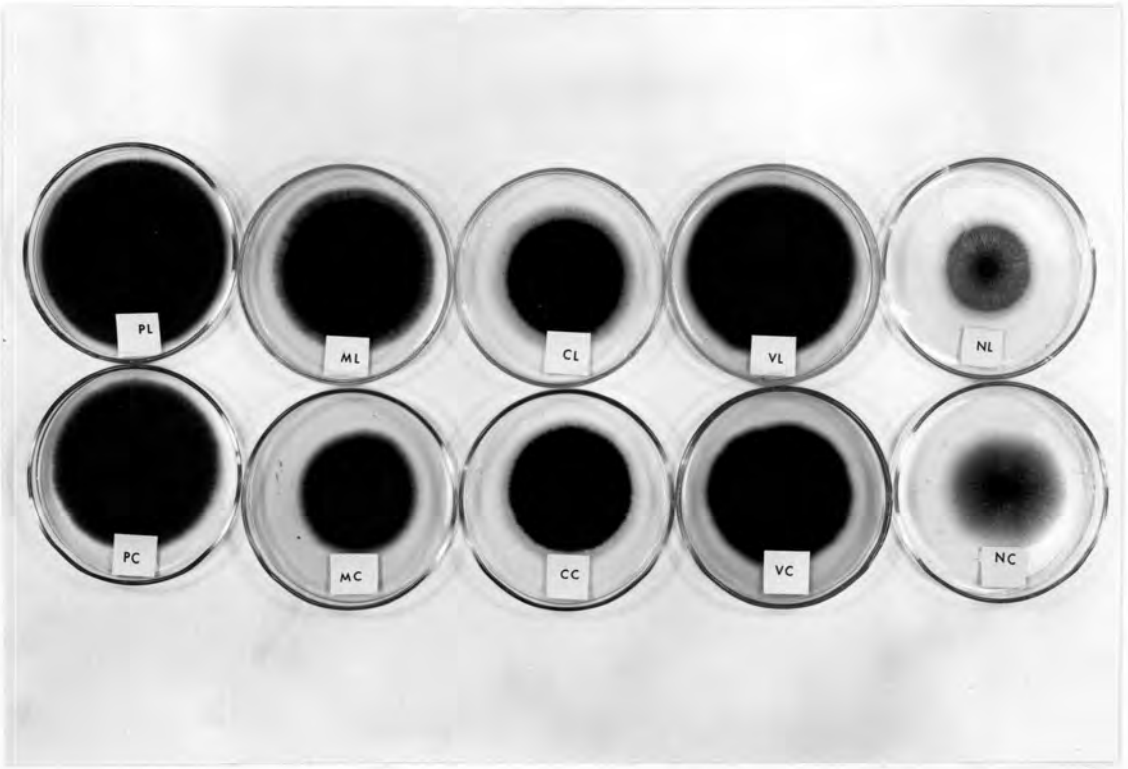

Figure 10. Effect of different media and near ultraviolet radiation on the growth of Helminthosporium dematioideum.

P, Potato dextrose agar; M, Malt agar; C, Czapek agar; V, V-8 juice agar; N, nutrient agar; L, in first row indicates irradiated cultures; C, in second row non-irradiated cultures.

INTERACTION OF TEMPERATURE AND NEAR ULTRAVIOLET RADIATION ON SPORULATION: Most of the studies previously described were conducted at a constant temperature of 70° F. It was felt however, that the possibility of an interaction of temperature and radiation on sporulation could not be ignored. To determine this relationship, colonies (three replications per treatment) were grown in darkness on PDA in Pyrex Petri dishes for four days at temperatures 41, 59, 69, and 77° F, and then exposed for seven days to near ultraviolet radiation from two 20 watt BLB Black Light lamps at 32 cm. distance at these same temperatures. Following irradiation the colonies were incubated a further 24 hours in darkness. The number of conidia produced per 2 mm^2. was determined microscopically at 100X magnification. To maintain a constant output from the lamps even at the lowest temperatures, the lamps were mounted within an insulated box kept at a temperature of 70° F. The air within the box was warmed by two 25 watt incandescent lamps connected to a thermostat. A small fan was mounted within the box to keep the air circulating. Direct exchange of air between the lamp box and incubator was prevented by covering the open side of the box with a sheet of uncoated cellophane.

In all species exposed to near ultraviolet radiation, more abundant sporulation was obtained under irradiation than under darkness irrespective of temperature (Table 9). Temperatures however, had a significant effect on the abundance of sporulation in both irradiated and non-irradiated colonies, though the optimum temperature for sporulation varied among species.

Temperature had a marked effect on sporulation in H. dematioideum. The fungus sporulated profusely in darkness at 77° F (Table 9) while at 41 and 59° F there was no sporulation in darkness and only a few conidia were produced at 69° F. Greatest perithecial production by Pyrenophora teres was obtained in the 59-69° F range in irradiated cultures with apparent reduction of perithecial production at higher temperature. It was concluded that temperature affected both the production and size of perithecia in both irradiated and non-irradiated cultures of P. teres. Temperature was also an important factor in sporulation of H. dematioideum and H. oryzae both of which were able to achieve approximately the same degree of sporulation in dark grown colonies as in irradiated colonies incubated at lower temperatures.

INTERACTION OF pH AND NEAR ULTRAVIOLET RADIATION: H. dematioideum and H. siccans were grown on PDA buffered (after autoclaving) with potassium phosphate buffers to

Table 9. INTERACTION OF TEMPERATURE AND CONTINUOUS EXPOSURE TO NEAR ULTRAVIOLET RADIATION ON SPORULATION.

Species	Number of Spores Per 2 mm² (Mean)[1/]							
	41°F		59°F		69°F		77°F	
	Ultra-violet	Dark	Ultra-violet	Dark	Ultra-violet	Dark	Ultra-violet	Dark
H. dematioideum	0	0	0	0	569	26	1800	1100
H. oryzae	0	0	820	421	975	342	716	510
H. sorghicola	0	0	103	13	345	106	413	116
H. setariae	0	0	8	0	0	0	0	0
H. victoriae	0	0	254	18	342	24	856	20
H. siccans	30	20	36	0	40	0	34	0
Pyrenophora teres	0	0	68	15	61	28	43	11

1/ Twelve day old colonies.

the following initial pH values: 5.0, 5.8, 6.2, 6.6, and 8.0. The buffered media were inoculated at the center with the plugs of mycelium obtained from the periphery of dark grown colonies and the plates were exposed at 70° F for seven days under continuous exposure to near ultraviolet radiation (eight 40 watt BLB Black Light lamps at 56 cm. 120 F.C.) and in darkness. After seven days exposure the plates were transferred to darkness and incubated a further 24 hours at 70° F, the amount of sporulation was then measured microscopically at 100X magnification.

H. dematioideum and H. siccans grew over a range of pH 5.0 - 8.0. At all pH values irradiation increased sporulation. A pH of 5.8 appeared to be near the optimum for sporulation in irradiated colonies for both species (Table 10). Sporulation was considerably reduce in colonies grown at pH 8.0. H. siccans did not sporulate at all in darkness and it appeared that pH had no effect on the sporulation of dark grown cultures of this species. It was concluded that conidia are stimulated by irradiation over a fairly wide range of hydrogen-ion concentrations but that pH of the medium had some effect on sporulation in the species studied.

Table 10. INTERACTION OF pH AND CONTINUOUS EXPOSURE TO NEAR ULTRAVIOLET RADIATION ON SPORULATION OF TWO SPECIES OF HELMINTHOSPORIUM.

Species		Number of Spores Per 2 mm^2 (Mean)[1/]				
		pH 5.0	pH 5.8	pH 6.2	pH 6.6	pH 8.0
H. dematioideum	Irradiated	500	686	539	420	289
	Dark	60	90	60	30	10
H. siccans	Irradiated	40	80	70	60	36
	Dark	0	0	0	0	0

1/ Nine days old colonies.

Effect of Irradiation on Spore Morphology

It was noticed in previous experiments that irradiation had affected the size of spores in certain of the species tested. Among these, Pyrenophora teres appeared to produce larger perithecia and H. victoriae larger conidia in irradiated colonies. To verify these observations experimentally, colonies were incubated under near ultraviolet Black Light lamps and in darkness. Four mm. discs of mycelium obtained from the periphery of colonies were transferred to the center of PDA plates and irradiated under continuous exposure to near ultraviolet radiation (eight 40 watt BLB Black Light lamps at 56 cm. distance, and 120 F.C.) for seven days at 70° F. Control plates were kept in light-proof containers. Three replications were used for each treatment. Following exposure, cultures were incubated at 70° F for an additional 24 hours and measurements were made of the diameters of perithecia in colonies of P. teres and the size of conidia in colonies of H. victoriae. Diameter of 100 perithecia and length and width of 100 conidia were measured at random in irradiated and non-irradiated colonies.

Perithecia in irradiated colonies of P. teres were almost twice the size of perithecia obtained from non-irradiated colonies (Table 11). In addition, conidiophores

in non-irradiated colonies were significantly longer than those in exposed colonies (Table 12). Spore size and septation of H. victoriae were also significantly affected by irradiation. Conidia in exposed colonies were approximately 1.25 times longer and showed more septation than conidia from non-irradiation colonies. Width of conidia in irradiated colonies also were greater than those in non-irradiated colonies. In addition to size differences, conidia from exposed colonies were also darker in color than conidia produced in colonies grown in darkness.

Table 11. EFFECT OF CONTINUOUS EXPOSURE TO NEAR ULTRAVIOLET RADIATION ON THE SIZE OF PERITHECIA OF PYRENOPHORA TERES.

Diameter of Perithecia (Microns)[1]	
Irradiated	Non-irradiated
156.3[2]	72.8[2]

1/ Mean of 100 measurements

2/ Irradiated vs. non-irradiated significantly different at 1 per cent level by Students' t test.

Table 12. EFFECT OF CONTINUOUS EXPOSURE TO NEAR ULTRAVIOLET RADIATION ON THE SIZE[1] OF CONIDIA AND CONIDIOPHORES OF *HELMINTHOSPORIUM VICTORIAE*.

Length of Conidiophores[2] (microns)		Length of Conidia[2] (microns)	
Irradiated	Non-irradiated	Irradiated	Non-irradiated
99.2	135.5	65.5	49.3

Width of Conidia[2] (microns)		Number of Septa/Conidia[2]	
Irradiated	Non-irradiated	Irradiated	Non-irradiated
10.8	10.0	7.29	5.27

1/ Irradiated vs. non-irradiated significantly different at 1 per cent level in all measurements by Students' t test.

2/ Mean of 100 measurements.

Effect of Monochromatic Ultraviolet Radiation on Reproduction of H. dematioideum

To elucidate the precise relationship of electromagnetic radiation to the induction of sporulation in one species of Helminthosporium, colonies of H. dematioideum were irradiated with monochromatic radiation of known wave lengths, intensity and duration. The apparatus and procedures used in these experiments were essentially the same as those reported by Leach for Ascochyta pisi (30).

A Bausch and Lomb diffraction grating monochromator (250 mm Model) with special high intensity quartz mercury arc lamp (Hanovia D-613C), was used as a source of radiation. The mercury arc lamp was mounted in a water-jacket housing through which tap water circulated continuously at approximately 50° F to remove excess heat from the lamp. A constant voltage transformer was used for the power supply.

Intensity was measured with a compensated thermopile (Kipp and Zonen E20) and a galvanometer (Kipp and Zonen "Portable Galvanometer" A70) calibrated by the manufacturer for absolute measurements. The lamp was turned on 15 minutes before actual measurements in order to obtain a fairly constant intensity. To eliminate infra-red background radiation emitted from the

monochromator housing, a cylindrical water jacket (circulating tap water at approximately 50° F) was inserted between the exit lens and the thermopile. By using a water-jacket, the background radiation was kept constant and the galvanometer could be zeroed. To measure intensity of radiation (1) the thermopile with its slit opened to 2 mm. was positioned in the monochromator's plane of focus and adjusted until a maximum reading was obtained on the galvanometer, (2) The desired intensity of radiation was obtained by adjusting the entrance slit vee-slide. Radiation intensity was measured prior to each irradiation series.

Colonies were irradiated at 3663 Å, 3131 Å, 2537 Å and 2378 Å at an intensity of 100 μ w/cm^2 and also at 3663 Å and 3131 Å at 300 μ w/cm^2. A band-width of 132 Å was used in all instances. An automatic irradiation apparatus (30) (Figure 11), was used for long exposures of 10-20,000 seconds. Colonies were grown in small plastic containers (26 x 5 x 1.8 cm). Fifteen ml. of two per cent potato dextrose agar was poured into each container and when solidified was inoculated with 4 mm. diameter discs of mycelium obtained from the periphery of dark grown colonies. The tops of the containers were covered with sterilized uncoated cellophane (2 microns thick) to prevent contamination.

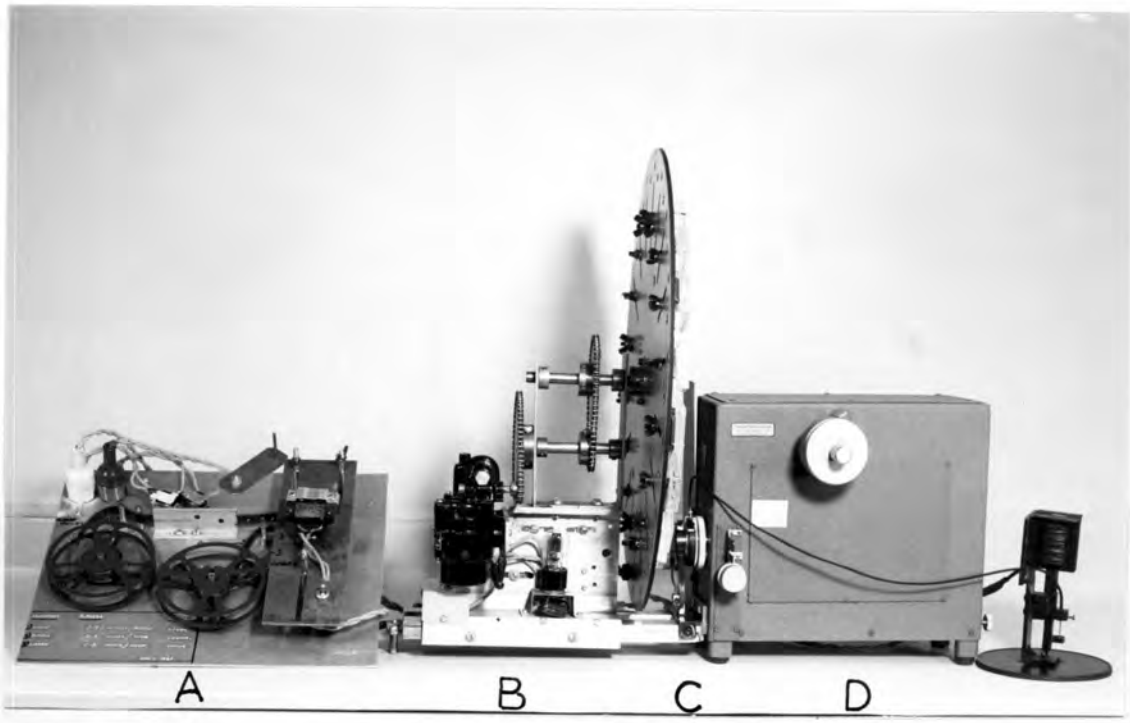

Figure 11. An automatic exposure apparatus for use with monochromatic radiation. (Through courtesy of C. M. Leach, Oregon State University).

A. Timing mechanism
B. Rotating disk mechanism
C. Camera shutter
D. Monochromator

The percentage transmission through uncoated cellophane was determined for wave lengths between 2378 Å - 5461 Å using the monochromator and thermopile and this information was used to adjust the intensity of radiation needed at colony surface. Colonies were grown in darkness for five days at 70° F prior to irradiation. To irradiate over short exposures (1/50 - 5 seconds), colonies were mounted on a mechanical stage in front of the monochromator's exit lens. The lamp was turned on 15 minutes before irradiation to obtain constant intensity. The different exposures selected ranged from 1/50th of a second to 20,000 seconds, and intensities were 100 μ w/cm^2 and 300 μ w/cm^2. After irradiation the containers were incubated for 24 hours at 70° F and then the numbers of spores produced per 1 mm^2 area were counted under a dissecting microscope at a magnification of 40X. Three replications per exposure were used for exposures ranging from 1/50 - 1000 seconds, while longer exposures were replicated only twice.

The effect of monochromatic radiation on sporulation was as follows (Figure 12):

Wave length 2378 Å: At an intensity of 100 μ w/cm^2 moderate conidial formation started at an exposure of 20 seconds and the greatest number of conidia were

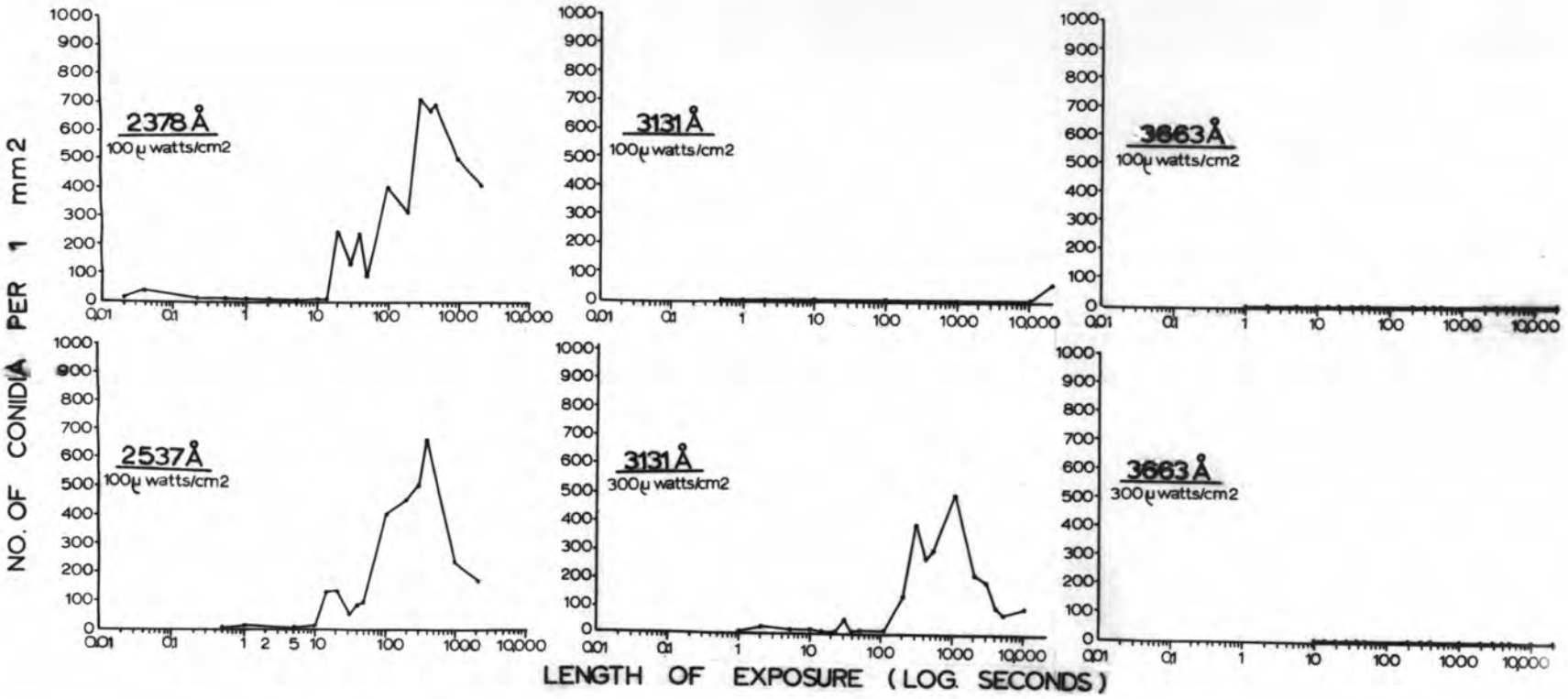

Figure 12. Effect of monochromatic ultraviolet radiation on reproduction of H. dematioideum.

obtained at an exposure of 300 seconds. As the exposure increased beyond 300 seconds, the number of conidia gradually decreased (Figure 12).

Wave length 2537 Å: At 100 μ w/cm^2, conidial production started after a minimum exposure of 15 seconds with maximum sporulation occurring after an exposure of approximately 500 seconds. Long exposures (1000 seconds and longer) caused an inhibition of sporulation (Figure 12).

Wave length 3131 Å: At an intensity of 100 μ w/cm^2, negligible sporulation occurred when colonies were exposed for 1/2 to 10,000 seconds. Only at an exposure of 20,000 seconds was there an indication of increased sporulation. At an intensity of 300 μ w/cm^2, however, induction of sporulation started after an exposure of 300 seconds.

Wave length 3663 Å: At an intensity of 100 and 300 μ w/cm^2, no sporulation was induced even after a maximum exposure of 20,000 seconds.

DISCUSSION

This study was originally started with the hypothesis that the species of a genus might respond similarly to radiation. To test the hypothesis, 23 species of Helminthosporium were selected and subjected to different radiation treatments. Sporulation was induced or increased when 13 of these species were irradiated under near ultraviolet radiation. This suggests that near ultraviolet radiation might be an important factor influencing sporulation.

Several studies have shown that visible light, particularly the blue region of the spectrum, stimulates reproduction. However, from the results of this study it is apparent that near ultraviolet radiation is more effective in inducing sporulation than longer wave lengths. Shorter wave lengths of ultraviolet radiation also induced reproduction when cultures were exposed to a germicidal lamp, though relatively short exposures also caused lethal or inhibitory effects. In contrast, there was no indication of lethal or inhibitory effects when colonies were subjected to long exposures to near ultraviolet radiation. It is quite possible that higher dosages of near ultraviolet radiation might also inhibit reproduction. Indeed, monochromatic irradiation studies with the fungus

Ascochyta pisi (29) supports this view, at least for the shorter wave lengths of near ultraviolet radiation. These studies indicated that it is not easy to compare the biological effects of qualitatively different polychromatic radiation because of the technical difficulties of measuring and standardizing intensities involved. If one is to critically compare the qualitative and quantitative effects of radiation on fungus reproduction, it is imperative that sources of monochromatic radiation of known intensity be used.

Among the 23 species, H. tritici-vulgaris, H. pedecellatum, H. gramineum, H. velutinum, H. sigmoideum, and H. rostratum did not sporulate in response to visible or ultraviolet radiation even when irradiated on different media and at different temperatures. Increase in pigmentation and inhibition of growth did occur when these species were exposed to a germicidal lamp. It is not possible, however, to state that these species are inherently incapable of sporulation for lack of sporulation may have resulted from absence of a critical factor or combination of factors not included in this study. Another possibility for the lack of reproduction in irradiated colonies may have been that these species had lost their ability to sporulate after years of

artifical culture. In retrospect, it would have been better to have started with newly isolated cultures.

H. nodulosum, H. monoceras, H. catenarium and H. sativum sporulated as well under irradiation as under continuous darkness. Thus "light" seems to have had no measurable effect on the initiation or stimulation of conidial production in these species. Their response corresponds to that reported for H. sativum (28, p. 158).

H. sacchari, H. vagans, H. torulosum, H. alii, H. carbonum, H. turcicum, H. dematioideum, H. oryzae, H. sorghicola, H. setariae, H. victoriae, H. siccans and Pyrenophora teres showed moderate to heavy sporulation when exposed continuously to near ultraviolet radiation, while under darkness sporulation ranged from none to moderate. Repeated short exposures of near ultraviolet radiation induced greater reproduction than continuous near ultraviolet or continuous visible radiation in H. dematioideum, H. victoriae and Pyrenophora teres. Possibly as suggested by Barnett and Lilly (2, p. 87) two enzyme systems are involved in the synthesis of the substance or substances necessary for conidium formation. For the first step, light appears to be essential, while the second is inhibited by light. Thus when the above mentioned three species were exposed to periods of light and darkness, conidium formation was greater than

under continuous exposure. Perhaps certain species require continuous exposure to radiation for heavy sporulation while others do not.

When wave lengths in the ultraviolet and blue region of the spectrum were removed from daylight fluorescent lamp radiation, sporulation generally was less than in colonies grown under near ultraviolet radiation. H. dematioideum sporulated only sparsely or not at all under visible radiation from which blue and ultraviolet radiation had been filtered, indicating that in this species ultraviolet radiation plays an important part in the induction of sporulation. However, in a few species visible radiation minus ultraviolet induced sporulation equally as well as near ultraviolet radiation. It is not possible to make a valid comparison because the visible radiation was at approximately five times the intensity of the near ultraviolet radiation. There is evidence from monochromatic radiation studies (29) that some effect on sporulation at one wave length can be duplicated at another longer wave length by increasing the dosage.

Brief exposures to a germicidal lamp induced sporulation in many of the species tested (Table 3). Among these the induction of sporulation in H. setariae indicated that short exposures to a germicidal lamp at

a particular intensity can induce sporulation in species which do not sporulate readily when irradiated at longer wave lengths. In general, high intensities and long exposures under the germicidal lamp reduced or inhibited sporulation.

The shorter wave lengths of radiation emitted by germicidal lamps are qualitatively atypical of the ultraviolet radiation received by fungi in nature. However, in its reproductive processes H. dematioideum was able to use short wave lengths more efficiently than the longer wave lengths. Thus at an intensity of 100 μ w/cm^2, the exposure necessary to induce sporulation at 3131 Å was approximately 600 times that required to induce the equivalent amount of sporulation at 2378 Å. When a higher intensity of radiation (300 μ w/cm^2) at 3131 Å was used, the same amount of sporulation could be achieved as by irradiation at 2378 Å at an intensity of 100 μ w/cm^2. It would seem therefore, that in nature, where far ultraviolet would normally be unavailable, this fungus must respond to near ultraviolet radiation at a greater intensity than was generally used in this study.

In addition to radiation, composition of media also influenced sporulation of irradiated colonies. The reasons for the interaction of substrate and irradiation are not known and require further work.

Temperature also modified the influence of radiation on sporulation. Though not all the species tested showed similar responses, in general, temperatures ranging from 59° F - 77° F were optimum for growth and sporulation in irradiated cultures.

Effect of irradiation on radial growth differed however, in many of the species tested growth under near ultraviolet was greater than in darkness. This indicates that physiological reactions other than spore formation also are affected by near ultraviolet radiation. Exposure of colonies to the shorter wave lengths of ultraviolet radiation mainly emitted by the germicidal lamp caused inhibition of growth at the higher dosages. There appeared to be little or no correlation between maximum growth and maximum sporulation, or vice versa.

In addition to inducing reproduction, it was also observed that ultraviolet radiation affects spore morphology and pigmentation of irradiated cultures. The density of pigmentation in irradiated colonies depended upon wave length, intensity of radiation and length of exposure. Fungicidal action of higher intensities, particularly at the shorter wave lengths, resulted in death and collapse of the aerial mycelium. Near ultraviolet profoundly affected the pigmentation, size and septation of conidia in H. victoriae, and size

of perithecia of Pyrenophora teres, and it is apparent from these studies that irradiation and interacting factors such as nutrition and temperature, affect the reproductive, mechanism, growth, colony characters, pigmentation and spore morphology. Since species identification is largely based on combinations of these characters, these results suggest the need for a new approach to the methods of identification and classification of the members of this genus, and possibly other fungi.

Finally, it was concluded that the original hypothesis that all species of a genus might react similarly when subjected to radiation, could not be supported experimentally, and was therefore not valid.

SUMMARY

1. Twenty-three species of Helminthosporium were exposed to visible and ultraviolet radiation to study the effect on sporulation. Although sporulation was induced in a number of species, the radiation requirements necessary to induce reproduction varied from species to species.

2. H. tritici-vulgaris, H. pedicellatum, H. gramineum, H. velutinum, H. sigmoideum and H. rostratum did not sporulate at all when exposed continuously either to near ultraviolet radiation or to visible radiation. In addition, exposure to a germicidal lamp at different intensities for different length of time failed to induce sporulation. Irradiation of colonies on different media and at different temperatures was also tried without positive results.

3. H. nodulosum, H. monoceras, H. catenarium and H. sativum sporulated equally well whether exposed to radiation or grown in darkness. Visible and ultraviolet radiation seemed to have had no appreciable effect on the reproduction of these species.

4. Sporulation was initiated or increased when H. dematioideum, H. oryzae, H. sorghicola, H. setariae, H. victoriae, H. siccans, H. sacchari, H. vagans, H. torulosum, H. alii, H. carbonum, H. turcicum, and

Pyrenophora teres were exposed continuously to near ultraviolet radiation. Under darkness, sporulation ranged from none to moderate.

5. Continuous visible radiation without ultraviolet did not affect conidial production in H. dematioideum. However, in a few species visible radiation minus ultraviolet induced sporulation equally as well as near ultraviolet radiation. Because of intensity differences in these experiments, valid comparisons between the effectiveness of visible and ultraviolet radiation could not be made.

6. Repeated short exposures (12 hours of light alternated with 12 hours of darkness) to near ultraviolet radiation induced greater reproduction than continuous near ultraviolet or continuous visible radiation in H. dematioideum, H. victoriae and Pyrenophora teres.

7. When H. dematioideum, H. setariae, H. siccans and P. teres were exposed (from 5 minutes to 1 hour) to a germicidal lamp at 163 μ w/cm^2 once each day for three days, a variety of responses were obtained. In H. dematioideum, H. setariae and H. siccans sporulation was greatest after a 5 minute exposure, while in P. teres maximum perithecial production occurred after a 30 minute exposure. Long exposures and higher intensities tended to inhibit sporulation in those species tested.

8. Growth responses of irradiated cultures differed. In some of the species tested, growth under near ultraviolet was greater than in the cultures grown in darkness. There were indications that radiation from the germicidal lamp suppressed growth and that the amount of suppression increased with increase in length of exposure. There appeared to be little or no correlation between maximum growth and maximum sporulation or vice versa.

9. Continuous near ultraviolet radiation affected the morphology and pigmentation of conidia of Helminthosporium victoriae and the size of perithecia of Pyrenophora teres.

Conidia of H. victoriae produced in irradiated colonies were larger in size and had greater numbers of septa than the conidia from non-irradiated colonies. Conidia produced in exposed colonies were also darker in color than those obtained from colonies grown in darkness. Perithecia of P. teres were approximately 1.5 times larger in irradiated colonies than in dark-grown colonies.

10. Reproduction of H. dematioideum, H. victoriae and P. teres was most abundant in irradiated colonies on five different media. In general the differences in the effect of nutrition on reproduction was most

pronounced in colonies grown in darkness. There were also differences in irradiated colonies on the various media but these were not so pronounced.

11. Temperature had a significant effect on the abundance of sporulation in both irradiated and non-irradiated colonies. Optimum temperature range (59-77°F) for sporulation varied among species.

12. Conidial production was stimulated by irradiation of colonies (H. dematioideum, H. siccans) grown over a fairly wide range of pH values. Acidity of the medium had some effect on profuseness of sporulation.

13. Monochromatic radiation studies with Helminthosporium dematioideum indicated that shorter wave lengths were more efficient in inducing conidial production than the longer wave lengths. Thus at 3131 Å (100 μ w/cm^2) the minimum exposure necessary to induce sporulation was approximately 600 times that required to induce the equivalent amount of sporulation at 2378 Å at the same intensity.

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