EFFECTS OF AUXINS AND LIGHT ON GROWTH OF THE FUNGUS *PHYMATOTRICHUM OMNIVORUM*

by

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ABSTRACT

The effects of white light and selected auxins and antiauxins on the vegetative growth of Phymatotrichum omnivorum (Shear) Duggar were studied in an effort to determine whether or not these influenced growth of an organism with non-cellulosic cell walls. Light intensities of up to 700 foot candles, the auxins indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and the antiauxins trans-cinnamic acid (TrCA) and maleic hydrazide (MH) were used. Radial vegetative growth was inhibited by concentrations of 2,4-D and TrCA equal to or greater than 21 x 10^{-5}M and by continuous white light. IAA concentrations of 7 x 10^{-5}M stimulated radial growth. Interactions between pairs of these chemicals were reflected in changes in radial growth and macroscopic appearance. Illuminated cultures grew in concentrations of 2,4-D which completely inhibited growth. The interactions of light and auxins with P. omnivorum indicate that the action of auxins on fungi without cellulosic cell walls may be similar to the action of auxins in higher plants. This approach to the possible role of auxins as fungal growth regulators has not been fully explored and several suggestions for further study have been presented.

INTRODUCTION AND LITERATURE REVIEW

Visible light and auxins influence the growth of higher plants. Although precise mechanisms have not yet been fully explained, light is known to photo-oxidize indole-3-acetic acid (IAA) and is believed to affect the translocation and/or synthesis of this compound. IAA, in turn, affects the elasticity, plasticity and division of the cellulosic cell walls of green plants (14).

The cell walls of many fungi have been shown to contain no cellulose. Instead, chitin is the primary wall constituent of the Zygomycetes, Ascomycetes and Basidiomycetes. The Oomycetes have cellulosic cell walls (5). Both cellulose and chitin have been found in the cell walls of Rhizidomyces sp. (11) and Ceratocystis ulmi (23).

Ergle and Blank (9) reported the presence of chitin and the absence of cellulose in the cell walls of Phymatotrichum omnivorum (Shear) Duggar. Although their techniques of chemical analysis are considered, by current investigators, to be inconclusive for small quantities of chitin or cellulose (15, 11), they are probably adequate for macrochemical quantities. The growth habits and morphological characteristics of this imperfect fungus indicate that it is probably related to members of the Basidiomycetes.

Gruen (13) has compiled a list of fungi reported to be affected by auxins and recent reviewers (13, 19) have discussed the

production and oxidation of IAA by this group of organisms. Those investigating the effects of auxins on growth have usually confined themselves to a single concentration or to a range of widely separated concentrations. In 1952, Banbury (2) disputed the contention that auxins influence growth of fungi based on his observations of the responses of Phycomyces blakesleeanus. Since then Cochrane (5), Carlile (4) and Gruen (12) have supported his views.

Reviews (4, 5, 15, 17, 19) of the effects of light and IAA on fungi have primarily discussed sexual and asexual reproductive structures, usually the sporangiophores of Phycomyces blakesleeanus. These sporangiophores have enabled investigators to obtain precise quantitative data comparable to that obtained by those using Avena coleopties (e.g., 7, 20). For the present, however, data obtained with one fungus cannot be generalized to apply to other fungal structures or taxonomic groups.

Response to visible light is varied and the only obvious generalizations are that fungi are most sensitive to the shortest wavelenghts (4) and that high intensities tend to reduce vegetative growth or to stop it completely. Light is known to affect the color, size, texture and/or vigor of the mycelium, and the size and shape of reproductive structures (4, 18). It is necessary, in some cases, for completion of the life cycle (5).

Observers noting the effects of light on vegetative growth of fungi frequently have not mentioned such conditions as light intensity, quality, or duration (e.g., 6). While most investigators have used a

clearly defined artificial growth medium, others have not, and few have tried more than one medium. Reports of Hall (14), Dickson (8), and Carlile (4) suggest that the nature of the growth medium can reverse the light-dark growth responses of Sclerotinia fructigena. Growth of Pilobolus kleinii is similar under light and dark conditions if ferrichrome is used as the iron source in the medium. However, if hemin is substituted, growth of the fungus is severely limited by light (4). It has also been shown that the ash (5) and polysaccharide (3) composition of the cell wall of Neurospora varies as the growth medium is changed. Do these facts further support the hypothesis that cell wall composition determines the light-dark growth response? Do auxins in the medium affect wall composition either directly or indirectly? First, we must know whether or not auxins influence the growth of fungi.

If auxins do influence hyphal extension, they should do it on all media which support growth. Auxins added should have no effect at very low concentrations and should inhibit growth at higher concentrations. IAA, the natural auxin most abundant in higher plants, is photo-oxidized by visible light. If chemically related compounds are responsible for growth regulation in fungi, then fungal auxins might also be photo-oxidized by visible light. A synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), which is not photo-sensitive, might, therefore, be able to replace the auxin destroyed by light. If so, then fungal growth would occur in light in concentrations of 2,4-D which, due to competitive inhibition, do not support growth in the dark. If IAA and 2,4-D have a

cumulative effect, it would be further verification that auxins affect fungal growth.

Auxin production is enhanced, in some fungi, by the addition of compounds such as tryptophan which are considered to be precursors of IAA.

Gruen (13) observed that the one factor common to reported cases of stimulation of vegetative growth by IAA was that promotion occurred on media which supported poor growth. It is possible that this happened because a factor necessary for the optimum production of growth regulating compound(s) was either absent, or present in very limited quantities.

If fungal growth is regulated by auxins, antiauxins should inhibit growth and a combination of auxins and antiauxins might nullify the effects of each other. However, maleic hydrazide (MH), is a special case among compounds classified as antiauxins. It inhibits growth in higher plants by preventing division of the cellulosic cell wall (16) rather than by competition with auxins for active sites. Since fungi grow by the extension of coenocytic hyphal tips and their cell walls may contain no cellulose, the existence of a mechanism similar to that of higher plants is questioned.

The purpose of this study was to observe some of the effects of light, auxins, and antiauxins on the vegetative growth of Phymatotrichum omnivorum. In addition, an attempt was made to determine the cell wall composition of this fungus.

METHODS AND MATERIALS

Two Phymatotrichum omnivorum isolates (University of Arizona, Numbers 1 and 34) were used. After isolation from cotton and alfalfa, respectively, the fungus was maintained on #70 medium at 25 to 30° C (Table I). Stock cultures used in these experiments had been on #70A medium (Table I) a minimum of three weeks before use and had undergone at least two transfers.

Preliminary testing indicated that diameter and type of growth would be similar on liquid and agar media. Therefore, unless otherwise stated, the fungus was grown in approximately 20 ml liquid medium in 100 x 10 mm Kimax petri dishes. The reported optimum temperature for vegetative growth of P. omnivorum is 28°C (9, 10). These reports were confirmed and all trials were run at 28°C unless otherwise stated. All treatments were replicated at least five times.

Inoculum for each experiment was the same age and incubated in 100 x 10 mm petri dishes for at least 10, but not more than 20 days. Agar pieces 5 mm square were cut with parallel scalpels and set in the center of dishes containing fresh growth medium. Only mycelium from near the center was used from cultures less than two weeks old.

Statistical methods, including analysis of variance and Duncan's multiple range test for difference of means, were used in the analysis of data obtained (22).

Table I. Growth media used for Phymatotrichum omnivorum

#70 medium (10)	
Glucose	40.00 g
NH_4NO_3	1.18
$MgSO_4 \cdot H_2O$	0.75
KCl	0.15
K_2HPO_4	0.492
KH_2PO_4	0.254
H_2O	to one liter
#70A medium	
#70 medium +	
$ZnSO_4 \cdot 7H_2O$	0.0110 g
$Fe_2(SO_4)_3$	0.0090
$MnSO_4 \cdot 7\ H_2O$	0.0077
#70C medium	
#70 medium +	
ZnEDTA	0.0078
Fe Citrate	0.0113
$MnSO_4 \cdot 7\ H_2O$	0.0077

Media were adjusted to pH 6.8 with KOH.

Agar (20 g/l) was added for solid medium.

Apparatus for light studies consisted of a table equipped with two four-foot banks of fluorescent lights in a light proof room. Each bank contained two Sylvania 40 watt "cool-white" bulbs. Light intensity was measured in foot candles (fc) with a Weston Photometer (No. 125). Instruments of this type give relative measurements but indicate neither the quantity, nor the spectral characteristics of radiant energy reaching the photocell. This instrument did, however, make it possible to determine whether or not the same relative intensity was used in successive trials.

Light intensity was changed by adjusting the distance of the lights above the table. In all cases, lights were at least 14 inches from the cultures and distances of the lights did not affect the temperature inside the petri dishes (30°C).

Dark grown checks were put on the table and covered with sheets of aluminum foil arranged to form a light proof chamber. Cultures to be grown in the light were placed on top of the foil. A telethermometer cable was put in an empty petri dish under the foil so that the temperature could be checked without exposing dark grown treatments to light. Another cable was placed in a dish on top of the foil and meter readings showed that there was no temperature difference between light and dark treatments.

Replications of the same treatment were distributed so that some were in the center of the table while others were closer to the edge. Because of the reduced light intensity, no cultures were placed near the extreme edges or ends of the table.

To determine the reversibility of the effects of light, cultures were grown on agar medium in approximately 300 foot candles (fc) of continuous white light. After two weeks, several cultures and their dark grown checks were moved to a dark incubator. Remaining cultures from light and dark were used to inoculate fresh agar plates. These were put in both dark and light conditions so that mycelium was transferred from light to light, light to dark, dark to dark and dark to light. Observations were recorded after one and two weeks.

The effects of limited nutrient supply on light-dark growth responses were observed using distilled water and water agar as the growth media. Thus, the only nutrients available were in the agar block on which the inoculum was growing. Petri dishes 10 x 100 and 15 x 150 mm were used. Checks for both light and dark conditions were grown on #70A medium.

To determine the influence of several intensities of white light on radial growth, petri dishes were suspended at various distances beneath the lights to give relative intensities of 700, 450, 100, 70 and 40 fc of light. The longest and shortest diameters were measured ($\pm$1 mm) and total surface area was calculated from this information. On the fifth day all mycelial mats were rinsed with distilled water and placed on tared aluminum foil. Mats were dried for 48 hours at 61°C and weighed. This experiment was repeated using 700, 100 and 40 fc. Controls were grown in the dark. Since both dry weight and total area were obtained, it was possible to determine whether or not a correlation between dry weight and total area existed under the above conditions of light, temperature and nutrition.

The effects of diurnal light-dark regimes on vegetative growth responses were studied by attaching an automatic timing device to the lighting system. Photoperiods of 1, 12 and 23 hours were used and observations were recorded.

Solutions of IAA and 2,4-D from 10^{-3} to 10^{2} mg/l were used to determine the order of magnitude of the concentrations to which P. omnivorum would respond. These solutions were prepared by dissolving the chemical in a minimal amount of 95% ethyl alcohol and then adding a large quantity of distilled water at one time. These solutions were used in place of distilled water to prepare media. If stored, media were kept at 4°C for not more than 48 hours before use. Inoculum was grown on #70C medium (Table I).

The action and interaction of auxins, antiauxins and growth medium were studied using IAA, 2,4-D, trans-cinnamic acid (TrCA) and maleic hydrazide (MH). IAA concentrations used were 0, 10, 20, 30, and 40 mg/l corresponding to concentrations of 0, 7×10^{-5}, 14×10^{-5}, 21×10^{-5}, and 28×10^{-5} moles per liter. The other chemicals were mixed in equivalent molar concentrations. Combinations of chemicals and the concentrations used are tabulated in Table II. Media used were distilled water, #70A and #70C. The experiment was terminated after eight days.

Representative cultures were photographed using Kodak High Contrast film (f 5.6 for 2 seconds). The film was developed in D76 for five minutes.

Table II. Concentration and combinations of chemicals used in auxin experiment.

Treatment Number	Concentrations[1]			
	IAA	2,4-D	TrCA	MH
1	7	-	-	-
2	14	-	-	-
3	21	-	-	-
4	28	-	-	-
5	7	7	-	-
6	7	14	-	-
7	14	7	-	-
8	14	14	-	-
9	7	-	7	-
10	7	-	14	-
11	14	-	7	-
12	14	-	14	-
13	7	-	-	7
14	7	-	-	14
15	14	-	-	7
16	14	-	-	14
17	-	7	-	-

1. Concentrations x 10^{-5}M

(cont'd)

Table II. (Cont'd.)

Treatment Number	Concentrations			
	IAA	2,4-D	TrCA	MH
18	-	14	-	-
19	-	21	-	-
20	-	28	-	-
21	-	7	7	-
22	-	7	14	-
23	-	14	7	-
24	-	14	14	-
25	-	7	-	7
26	-	7	-	14
27	-	14	-	7
28	-	14	-	14
29	-	-	7	-
30	-	-	14	-
31	-	-	21	-
32	-	-	28	-
33	-	-	7	7
34	-	-	7	14
35	-	-	14	7
36	-	-	14	14
37	-	-	-	7
38	-	-	-	14

(cont'd)

Table II. (Cont'd.)

Treatment Number	Concentrations			
	IAA	2,4-D	TrCA	MH
39	-	-	-	21
40	-	-	-	28
41	-	-	-	-

The effects of light on P. omnivorum grown in four concentrations of 2,4-D (0, 11, 23 and 45 x 10^{-5}M) were observed at one intensity of white light, approximately 400 fc.

To determine the cell wall composition, approximately one kg (wet weight) mycelial mats were grown in Fernbach flasks. The cell walls were cleaned according to the method outlined by Fuller and Barshad (11). Briefly, the method consisted of boiling the chopped mycelium in 20% KOH until microscopic examination showed it to be free of cell contents. It was then rinsed in boiling water until the rinse water had a neutral pH. Chitosan which might have formed was removed by boiling the mycelium in 2% acetic acid and the pH was again returned to 7.0 by rinsing with distilled water. Reagents and mycelium were separated by centrifugation (approximately 100 g for 15 minutes), thus eliminating the possibility of introducing foreign material.

To analyze the material, an x-ray diffraction pattern using the Cu α-line was obtained. The pattern was recorded with a DeBye camera using exposure times of 30 and 60 minutes. Another sample of material was treated with Schweitzer's Reagent (24) to check chemically for the presence of cellulose.

RESULTS

Mycelial mats of Phymatotrichum omnivorum, grown on #70A medium, had more aerial hyphae and denser growth, but not always more radial area than those grown on H_2O. Whether liquid or agar culture was used, radial extension on #70A was the same as that on H_2O in all light experiments.

The controls in the auxin experiment did not, however, respond as those in the light experiments. The auxin controls grown on H_2O had three times the area of those grown on #70A or #70C media. Dark conditions used here and those in the light experiment differed only in the growth medium of the inoculum: #70C was used instead of #70A. In #70C, ferric citrate replaced ferric sulfate and zinc ethylenediaminetetraacetate (ZnEDTA) was used instead of zinc sulfate.

When all auxin treatments were averaged, radial growth on #70A did not differ significantly from that on #70C (Table III), but the area of cultures on each of these two media was significantly greater than that of those on H_2O. Consideration of the interactions of individual chemicals and pairs of chemicals with the media revealed that this was not always the case. Radial growth on H_2O differed significantly from that on #70C only when 2,4-D and TrCA were combined (Tables X and XII). The area of cultures grown on #70A was significantly greater than that on the other two media when TrCA was combined with IAA (Tables VI and VIII) and significantly less when TrCA was combined with MH (Tables XIV and XVI).

Table III. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance for total auxin experiment

Source	Degrees freedom	Sum of squares	Mean square	F
Treatment	40	20,656.25	516.41	7.05**
Media	2	4,021.64	2,010.82	27.44**
Treatment x Media	80	21,793.82	272.42	3.72**
Error	469	34,372.81	73.29	

** F exceeds 0.99

Table IV. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance; media x chemical x concentration

Source	Degrees Freedom	Sum of squares	Mean square	F
Media	2	289.48	144.74	2.71
Concentration	4	9,292.57	2,323.14	43.57**
Chemical	3	1,597.22	532.41	9.99**
Media x Concentration	8	5,985.80	748.23	14.03**
Media x Chemical	6	3,070.42	511.74	9.60**
Concentration x Chemical	12	1,923.76	160.31	3.01**
Media x Concentration x Chemical	24	568.33	23.68	.44
Error	224	11,943.87	53.32	

** F exceeds 0.99

Table V. Effects of the interaction of auxins and media on Phymatotrichum omnivorum; analysis of variance: 2,4-D x IAA x media

Source	Degrees freedom	Sum of squares	Mean square	F
Media	2	577.53	288.76	4.49*
IAA concentration	2	3,588.69	1,794.34	27.87**
2,4-D concentration	2	1,596.99	798.49	12.40**
IAA x 2,4-D	4	528.49	132.12	2.05
IAA x Media	4	1,251.44	312.86	4.86**
2,4-D x Media	4	1,589.38	397.39	6.17**
IAA x 2,4-D x Media	8	768.95	96.12	1.49
Error	103	6,630.99	64.38	

* F exceeds 0.95 value
** F exceeds 0.99 value

Table VI. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance: 2,4-D x TrCA x Media

Source	Degrees freedom	Sum of squares	Mean square	F
Media	2	1,404.43	702.21	11.10**
IAA concentration	2	1,492.96	746.48	11.80**
TrCA concentration	2	2,079.61	1,039.81	16.44**
IAA x TrCA	4	2,427.99	606.99	9.59**
IAA x Media	4	1,395.72	348.93	5.52**
TrCA x Media	4	3,218.43	804.61	12.72**
IAA x TrCA x Media	8	835.74	104.47	1.65
Error	106	6,706.26	63.27	

** F exceeds 0.99 value

Table VII. Effects of auxins and antiauxins on radial growth of *Phymatotrichum omnivorum*. Duncan's multiple range test: IAA x 2,4-D x media.

2,4-D Concentration ($\times 10^{-5}$M)	IAA Concentration ($\times 10^{-5}$M) 0	7	14	21	28
0	15.00 bcdef (BC)[1]	26.82 a (A)	8.78 defghijk (CD)	13.86 cdefg	7.66 efghijk
7	14.35 cdefg (BC)	17.61 bcd (B)	8.05 defghijk (CD)		
14	4.79 ghijk (D)	14.90 cdef (BC)	4.97 ghijk (D)		
21	3.04 ijk				
28	4.06 hijk				

Media	IAA Concentration	2,4-D Concentration
H_2O-10.36 (b)	0 - 11.01 (b)	0 - 16.64 (a)
70A-15.70 (a)	10 - 19.82 (a)	10 - 13.19 (b)
70C-11.60 (ab)	20 - 7.27 (b)	20 - 8.10 (c)

1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in parentheses ( ) indicate comparisons made on interactions of concentrations 0, 10 and 20.

Table VIII. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; Duncan's multiple range test: IAA x TrCA x media

TrCA concentration	IAA concentration 0	7	14	21	28
0	15.00 bcdef (CD)[1]	26.82 a (A)	8.78 defghijk (DE)	13.86 cdefg	7.66 efghijk
7	24.15 ab (AB)	17.15 bcde (BC)	13.04 cdefgh (CD)		
14	4.82 ghijk (E)	11.99 cdefghi (CDE)	9.09 defghijk (DE)		
21	7.28 efghijk				
28	0.49 k				

Media	IAA concentration	TrCA concentration
H_2O - 9.09 (b)	0 - 14.86 (a)	0 - 16.66 (a)
70A - 16.13 (a)	10 - 18.47 (a)	10 - 19.00 (a)
70C - 11.66 (b)	20 - 10.30 (b)	20 - 8.70 (b)

1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in parentheses ( ) indicate comparisons made on interactions of concentrations 0, 10, 20.

Table IX. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance: IAA x MH x Media

Source	Degrees freedom	Sum of squares	Mean square	F
Media	2	514.17	257.08	3.05
IAA concentration	2	1,294.76	647.38	7.69**
MH concentration	2	392.13	196.07	2.33
IAA x MH	4	1,234.18	308.54	3.67**
IAA x Media	4	2,753.72	688.43	8.18**
MH x Media	4	180.80	45.20	.54
IAA x Media x MH	8	889.84	111.23	1.32
Error	106	8,920.98	84.16	

** F exceeds 0.99 value

Table X. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance: 2,4-D x TrCA x Media

Source	Degrees freedom	Sum of squares	Mean square	F
Media	2	1,004.08	502.04	8.98**
2,4-D concentration	2	2,800.82	1,400.41	25.04**
TrCA concentration	2	3,361.26	1,680.63	30.05**
2,4-D x TrCA	4	520.03	130.10	2.32
2,4-D x Media	4	953.74	238.43	4.26**
TrCA x Media	4	2,295.71	573.93	10.26**
TrCA x Media x 2,4-D	8	1,257.20	157.15	2.85**
Error	101	5,649.05	55.93	

** F exceeds 0.99 value

Table XI. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; Duncan's multiple range test: IAA x MH x Media

MH concentration	IAA concentration 0	7	14	21	28
0	15.00 bcdef (B)[1]	26.82 (A)	8.78 defghijk (B)	13.86 cdefg	7.66 efghijk
7	15.93 bcde (B)	17.31 bcd (B)	15.47 bcdef (B)		
14	13.48 cdefgh (B)	14.09 cdefg (B)	10.93 cdefghij (B)		
21	10.41 cdefghij				
28	6.05 fghijk				

Media	IAA concentration	MH concentration
H_2O - 18.03 (a)	0 - 14.80 (ab)	0 - 16.64 (a)
70A - 13.99 (a)	10 - 19.36 (a)	10 - 16.24 (a)
70C - 13.72 (a)	20 - 11.72 (b)	20 - 12.80 (a)

1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in parentheses ( ) indicate comparisons made in interactions of concentrations 0, 10, 20.

Table XII. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; Duncan's multiple range test: 2,4-D x TrCA x media

TrCA concentration ($\times 10^{-5}$M)	2,4-D concentration ($\times 10^{-5}$M) 0	7	14	21	28
0	15.00 bcdef (BC)[1]	14.35 cdefg (B)	4.79 ghijk (C)	3.04ijk	4.06 hijk
7	24.15 ab (A)	18.89 abc (AB)	7.61 efghijk (CD)		
14	4.82 ghijk (D)	8.03 defghijk (CD)	1.37 jk (D)		
21	7.28 efghijk				
28	0.49 k				

Media	2,4-D concentration	TrCA concentration
H_2O - 7.90 (b)	0 - 14.88 (a)	0 - 11.01 (b)
70A - 10.81 (b)	10 - 13.68 (a)	10 - 17.10 (a)
70C - 14.22 (a)	20 - 4.52 (b)	20 - 4.74 (c)

1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in parentheses ( ) indicate comparisons made on interactions of concentrations 0, 10, 20.

Table XIII. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; analysis of variance: 2,4-D x MH x Media

Source	Degree freedom	Sum of squares	Mean square	F
Media	2	201.22	100.61	.99
2,4-D concentration	2	511.22	255.61	2.52
MH concentration	2	46.17	23.09	.23
MH x 2,4-D	4	1,470.72	367.68	3.63**
2,4-D x Media	4	5,443.13	1,360.78	13.42**
MH x Media	4	1,256.94	314.23	3.10*
2,4-D x Media x MH	8	2,323.00	290.37	2.86**
Error	102	10,340.99	101.38	

* F exceeds 0.95 level
** F exceeds 0.99 level

Table XIV. Effects of the interaction of antiauxins and media on the radial growth of Phymatotrichum omnivorum; analysis of variance: TrCA x MH x Media

Source	Degree freedom	Sum of squares	Mean square	F
Media	2	1,517.79	758.90	12.46**
TrCA concentration	2	1,749.84	874.92	14.37**
MH concentration	2	74.13	37.60	.62
TrCA x MH	4	1,776.66	444.17	7.29**
TrCA x Media	4	2,756.86	689.21	11.32**
MH x Media	4	428.39	107.98	1.77
TrCA x Media x MH	8	1,645.22	205.65	3.38**
Error	104	6,333.97	60.90	

** F exceeds 0.99 level

Table XV. Effects of the interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; Duncan's multiple range test: 2,4-D x MH x media

MH concentration ($\times 10^{-5}$M)	2,4-D concentration ($\times 10^{-5}$M) 0	7	14	21	28
0	15.00 bcdef (AB)[1]	14.35 cdefg (AB)	4.79 ghijk (B)	3.04 ijk	4.06 hijk
7	15.93 bcde (B)	19.95 abc (A)	15.90 bcde (A)		
14	13.48 cdefgh (AB)	16.26 bcde (A)	16.29 bcde (A)		
21	10.41 cdefghij				
28	6.05 fghijk				

Media	2,4-D concentration	MH concentration
H_2O - 15.80 (a)	0 - 14.80 (a)	0 - 11.01 (b)
70A - 12.94 (a)	10 - 17.17 (a)	10 - 17.26 (a)
70C - 15.07 (a)	20 - 12.25 (a)	20 - 15.32 (ab)

1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in parentheses ( ) indicate comparisons made on interactions of concentrations 0, 10, 20.

Table XVI. Effects of interaction of auxins and antiauxins on radial growth of Phymatotrichum omnivorum; Duncan's range test: TrCA x MH x media

MH concentration ($\times 10^{-5}$M)	TrCA concentrations ($\times 10^{-5}$M) 0	7	14	21	28
0	15.00 bcdef (BC)[1]	24.15 ab (A)	4.82 ghijk (D)	7.28 efghijk	0.49 k
7	15.93 bcde (B)	16.11 bcde (B)	7.26 efghijk (CD)		
14	13.48 cdefgh (BC)	13.70 cdefgh (BC)	15.99 bcde (B)		
21	10.41 cdefghi				
28	6.05 fghijk				

Media	TrCA concentration	MH concentration
H_2O - 15.85 (a)	0 - 14.80 (a)	0 - 14.88 (a)
70A - 8.86 (b)	10 - 18.08 (a)	10 - 13.10 (a)
70 C - 17.07 (a)	20 - 9.15 (b)	20 - 14.33 (a)

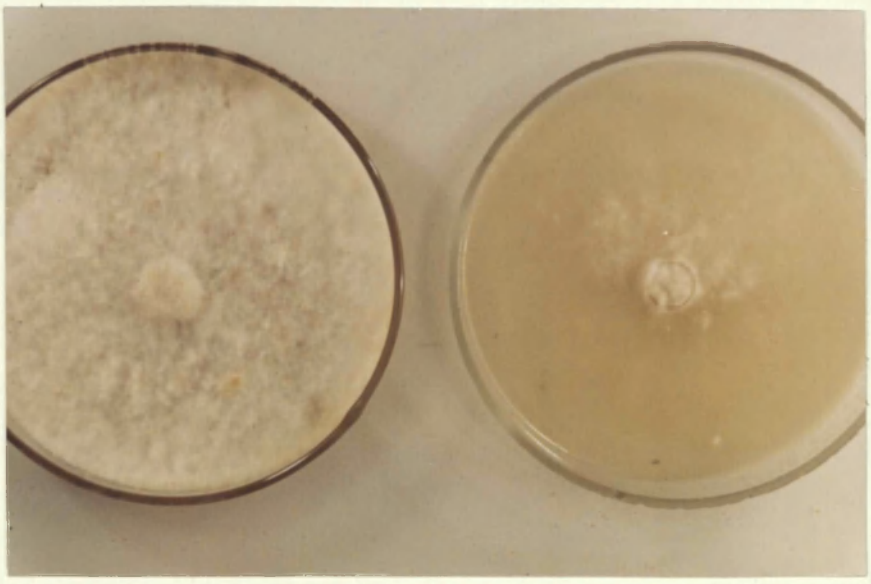
1. The same lower case letters following numbers indicate no significant difference at 0.99 level in comparison of all treatments. Letters in Parentheses ( ) indicate comparisons made on interactions of concentrations 0, 10, 20.

The combination of IAA and 2,4-D allowed more radial growth on #70A than on H_2O, but the area of cultures on #70C did not differ significantly from that on either of the other two. Each of the three media supported statistically the same radial growth in the presence of the combination of MH and 2,4-D (Tables XIII and XV). This was also true for the combination of MH and IAA (Tables IX and XI) and for the individual chemicals IAA, 2,4-D, TrCA and MH (Table IV) when these were considered separately.

Continuous illumination of cultures with white light was tried first with intensities of 700, 400, 100, 40 and 0 fc and then with 700, 100, 40 and 0 fc. Growth in 700 fc was light and delicate (Fig. 1). As light intensity decreased, the area, mass and flocculent growth of mycelial mats increased. However, cultures grown in 40 fc seemed to be more uniformly textured than those grown in the dark. In both trials, different intensities produced more significant variation in area than in dry weight (Table III). The first showed a general trend toward a decrease in growth as light intensity increased, but variation among replications decreased the significance of the results (Table XVII). The second trial (Table XVIII) showed a significant decrease in area for each increase in intensity. Dry weights of cultures grown in the light differed significantly from those grown in the dark, but not from each other. A correlation coefficient of 0.803 was obtained when total area and dry weight were compared.

P. omnivorum cultures which had been in continuous light for two weeks had sparse growth with little or no stranding. One week

Complete darkness 80 fc



Complete darkness 700 fc

Fig. 1. Comparison of vegetative growth in dark, 80 fc and 700 fc.

Table XVII. Effects of various light intensities on radial growth and dry weight of *P. omnivorum*: analysis of variance

	Degrees Freedom	Sum of Squares	Mean Square	F
Treatment (dry weight)	5	21,120.82	4,224.16	1.61
Error	26	68,282.90	2,883.99	
Treatment (total area)	5	3,621.35	724.27	3.70
Error	26	5,090.74	195.80	

Table XVIII. Repeat of experiment on the effects of several light intensities on radial growth: analysis of variance

	Degrees Freedom	Sum of Squares	Mean Square	F
Treatment (dry weight)	3	57,581.57	19,193.86	12.39**
Error	18	27,879.38	1,548.85	
Treatment (Total area)	3	2,712.05	904.17	111.21**
Error	18	146.32	8.13	

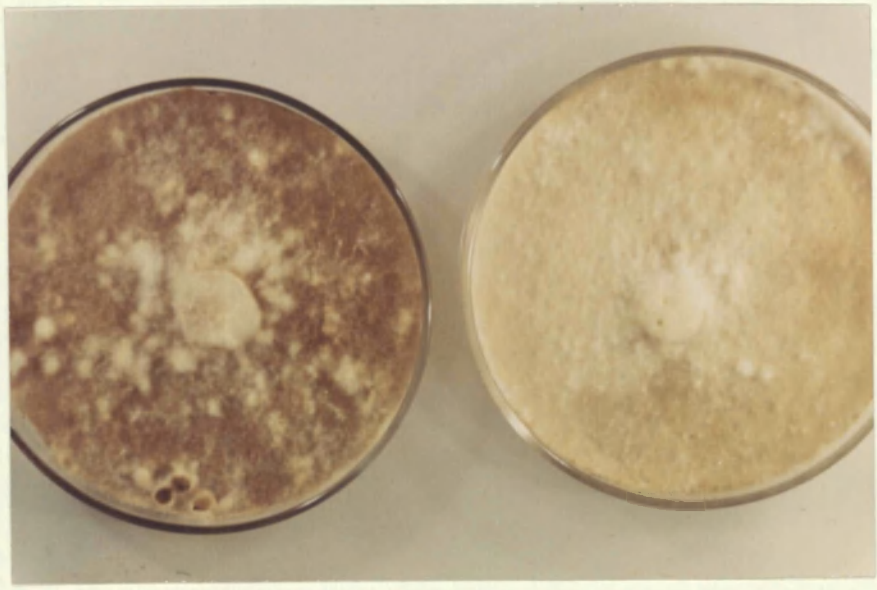
** F exceeds 0.99 level

after change to dark conditions, the mycelial mats had begun to fill in. By the end of the second week, they had lost their finely textured appearance and were covered with flocculent growth similar to that of the dark grown controls (Fig. 2).

When mycelial mats, grown at 400 fc, were used as inoculum, the new cultures began very slowly under both light and dark conditions. Those in light had so little growth after two weeks that a second sub-culture seemed impractical. Those transferred to dark conditions did, however, have sufficient growth for a second sub-culture.

Diurnal light-dark regimens did not produce concentric circles of growth. Instead, growth in 12 hour photoperiods at 350 fc was similar to that in continuous light of approximately 100 fc. It was difficult to distinguish between cultures grown in 23 and those grown in 24 hours of light. Likewise, response to complete darkness and to one hour of light (350 fc) was similar except that one hour of light seemed to stimulate more uniform growth than complete darkness.

Concentrations of 7×10^{-5}M IAA stimulated radial extension, but the area of cultures in 14, 21 and 28×10^{-5}M solutions did not differ significantly from that of the controls. 7×10^{-5}M 2,4-D supported radial growth which was statistically the same as that of the controls, but growth of cultures in concentrations of 14, 21 or 28×10^{-5}M was inhibited. Indications of additive effects of these two auxins were seen when they were combined in solution (Tables V and VII). The combination of 7×10^{-5}M IAA and 14×10^{-5}M 2,4-D reversed the stimulatory effect of 7×10^{-5}M IAA and the inhibitory effects of 14×10^{-5}M 2,4-D. Radial growth of resultant cultures did



Dark transferred to light. Light transferred to dark.

Dark transferred to light. Continuous dark.

Fig. 2. A comparison of mycelial mats transferred from dark to light conditions with those transferred from light to dark.

not differ significantly from those in 21 x 10^{-5}M IAA or the controls. Growth in solutions of these chemicals combined in concentrations of 14 x 10^{-5}M each was not significantly different from that in 28 x 10^{-5}M solutions of each chemical by itself (Fig. 3).

Four hundred fc of white light allowed P. omnivorum to grow in 23 x 10^{-5}M 2,4-D. Mycelial mats were of approximately the same size as those grown in the dark in concentrations of 12 x 10^{-5}M. There was no growth in the dark in concentrations of 23, 34 or 45 x 10^{-5}M or in the light in 34 or 45 x 10^{-5}M.

The two antiauxins elicited very different growth responses (Tables XIV and XVI). TrCA inhibited radial extension in 14, 21, and 28 x 10^{-5}M concentrations. Inhibition in 28 x 10^{-5}M solutions was complete except on H_2O medium where there was limited growth. Growth in concentrations of 7 x 10^{-5}M was not statistically greater than that of the controls, but the increase was such that it was only slightly less than the significant stimulation caused by this same concentration of IAA. Growth in treatments involving MH were not significantly different from that in the controls, but the lowest concentration supported significantly more growth than the highest. When combined with other chemicals MH has a leveling influence; 14 x 10^{-5}M 2,4-D (Table XV) and TrCA (Table XVI) inhibited growth and 7 x 10^{-5}M IAA (Table XI) stimulated it, but when each of these was combined with 7 or 14 x 10^{-5}M MH the area of the resultant cultures did not differ significantly from the controls.

None of the combinations of TrCA and IAA (Table VIII) produced radial growth which differed significantly from that of the controls.

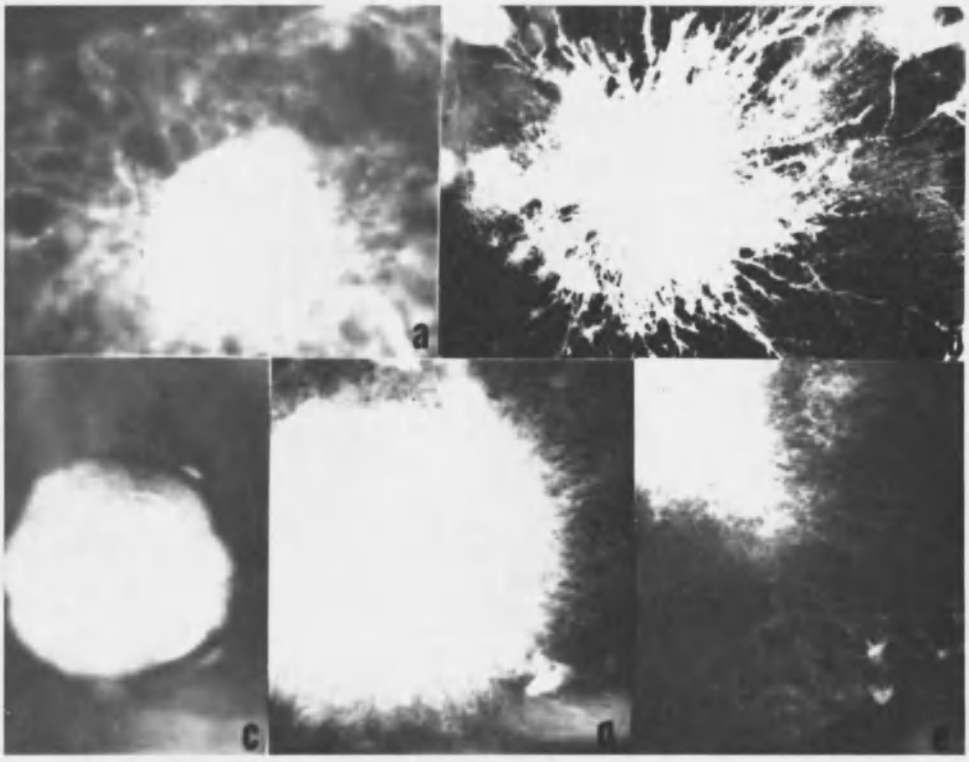


Fig. 3. Typical growth patterns of Phymatotrichum omnivorum:
a. Fine stranding is usual on H_2O medium.
b. Stranding of this type occurred in all cases when culture was almost dry.
c. Very fine textured mycelium frequently replaced control type in H_2O culture.
d., e., Uneven or rough circumference was more common than the smooth one of the control type, but occurred frequently.

However, some combinations of TrCA and 2,4-D did produce results which were significantly different from each other and from the controls. Just as combinations of 2,4-D and IAA inhibited growth, combinations of 2,4-D and TrCA (Table XII) in concentrations of 14 x 10^{-5}M each inhibited radial growth. The area of the resultant cultures did not differ significantly from that on 14, 21 or 28 x 10^{-5}M solutions of these chemicals individually.

MH was involved, along with IAA and TrCA in the stimulation of several unusual growth patterns. Single strands, which later branched, grew over the finely textured hyphae in treatments containing 14 x 10^{-5}M IAA combined with 7 and 14 x 10^{-5}M MH (Fig. 4c). A snowflake-like pattern (Fig. 4) appeared on H_2O medium either when MH concentration was 21 or 28 x 10^{-5}M, or when MH and TrCA were combined and the total concentration was 21 or 28 x 10^{-5}M.

X-ray powder analysis of the cell walls of P. omnivorum was inconclusive because the pattern obtained did not match those reported for either chitin, or cellulose, or for a combination of chitin and cellulose (11). Interplanar spacings and the relative intensities of these lines as they appeared in the x-ray powder diagram (Fig. 5) are reported in Table XVIII. The chemical test with Schweitzer's Reagent did not reveal any cellulose.

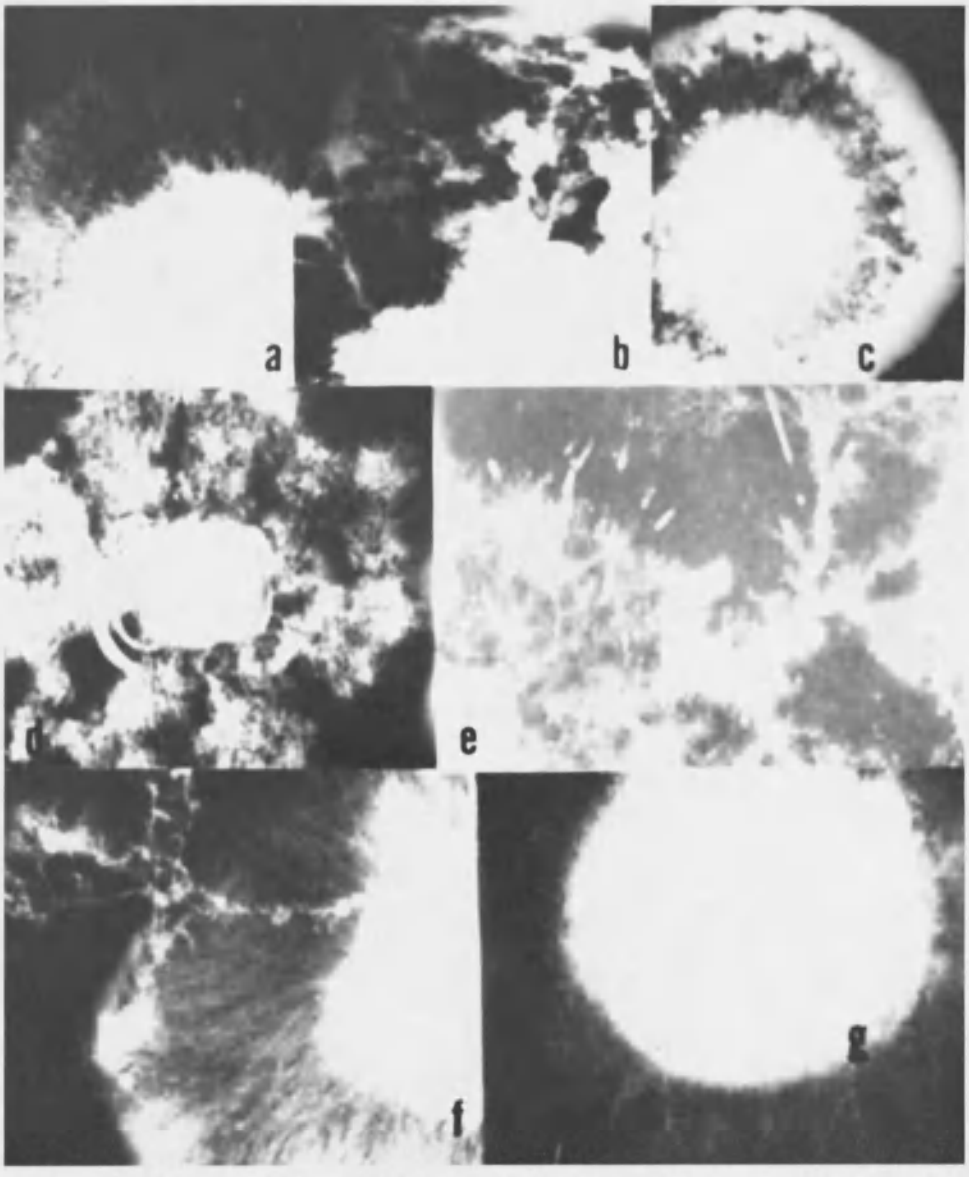


Fig. 4. Unusual growth patterns of Phymatotrichum omnivorum resulting from interaction with auxins:

a. (15-70C) concentric circles of growth appeared only in one plate.
b. (15-70C) same treatment as 4a except that plate was tipped so that much of it was dry.
c. Edges of mycelial mat were unusually thickened.
d. When it appeared, the snowflake pattern usually appeared in all replications.
e. (39-H_2O) unusual stranding, especially for H_2O medium.
f. (23-70C) an occasional strand over fine growth also appeared in 16-70A.
g. Abrupt change of density of growth and neatly serrated edges appeared only in this treatment.

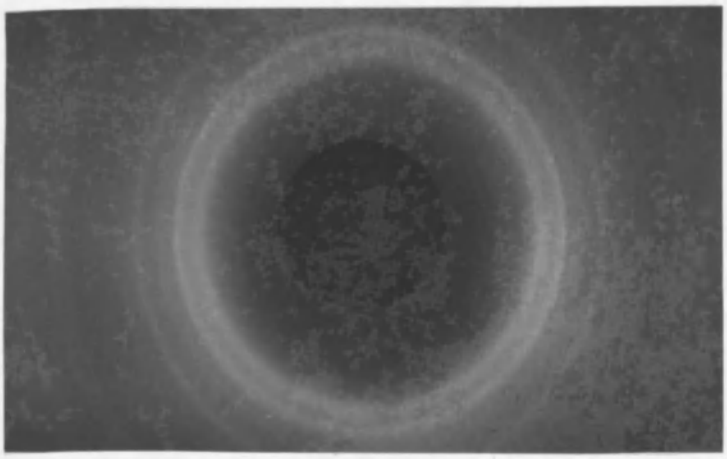


Fig. 5. X-ray powder diagram of Phymatotrichum omnivorum cell wall.

Table XIX. Interplanar spacings and intensities obtained by x-ray powder analysis of *Phymatotrichum omnivorum*

Distance in A	Relative intensity
5.4	10
4.2	9
3.8	3
3.4	7
2.6	3
2.5	3
2.35	3
2.3	3
1.63	2
1.56	2

DISCUSSION

The radial vegetative growth of Phymatotrichum omnivorum was affected by white light and the chemicals IAA, 2,4-D and TrCA. These factors also influenced the macroscopic appearance of mycelial mats, at times, in a similar manner. For example, the finely textured appearance of cultures grown on #70A and H_2O media in 700 fc of white light was similar to that of cultures grown in the dark on H_2O medium with concentrations of IAA equal to or greater than 14×10^{-5}M.

The fact that some combinations of chemicals and media seemed to duplicate effects of continuous light is of special interest. The inhibitory effects of higher light and auxin concentrations may or may not be related in fungi, but they are related in higher plants. The observations made here indicate that they could also be related in fungi.

Both macroscopic appearance and radial extension of P. omnivorum mycelial mats are affected by changes in light intensity, but dry weight seems to be affected primarily by the presence or absence of light. This suggests the existence of more than one response mechanism. One of these mechanisms could be related to the means of growth by extension of coenocytic hyphal tips or to the chitinous cell walls. Neither of these characteristics is shared with higher plants.

Whether dry weight or radial extension is used as the measure, vegetative growth of P. omnivorum is inhibited by white light of several

intensities. Similar observations have been reported for other fungi at least as far back as the middle 1800's (21). Unfortunately, it is impossible to compare light intensities used here to those used on other fungi because the foot candle is not a quantity which can be converted to, or compared with, intensity reported in energy units. Unless spectral characteristics of both light and meter are identical, two foot candle measurements cannot be compared.

The pattern of concentric circles of growth seen in some other fungi (e.g., Fusarium sp.) as a result of exposure to alternating periods of light and dark was not seen in P. omnivorum. This fungus does not ordinarily grow only at the external boundary of the mycelial mat. Instead, strands extend from the inoculum, branching as they proceed. At the same time, more finely textured strands and aerial hyphae fill in behind the growing front. This is done in such a manner that "light" and "dark" growth could be one on top of the other. More careful study is needed to determine precisely what happens to the "typical" growth pattern in conditions of alternating light and dark. Since growth in 700 fc appears to be only at the circumference of the mycelial mat, it would be interesting to see the effects of 12 or 16 hour photoperiods at this intensity.

The effects of light on P. omnivorum are at least partially reversible. It is difficult to determine whether or not the reversibility is complete because of the cumulative growth pattern described above.

Illuminated P. omnivorum cultures grew in concentrations of 2,4-D which did not support growth in the dark, suggesting that the 2,4-D might have replaced photo-oxidized natural auxin. This should be repeated using 2,4-D and other synthetic and natural auxins. Both dry weights and radial measurements should be statistically analyzed. The hypothesis that auxins affect fungal growth would be greatly strengthened if other fungi respond as Phymatotrichum did to combinations of 2,4-D and light.

In the dark, Phymatotrichum omnivorum responded to a narrow range of IAA concentrations. Seven x 10^{-5}M showed a significant (0.99 level) increase in area over that of the control. At the other end of the response spectrum, 35 x 10^{-5}M allowed little, if any, growth. Some previous studies have shown that other species of fungi do not respond to low concentrations of auxins, and are inhibited by high concentrations (13). The concentrations producing these effects varied with species and growth medium. A comparison of the concentrations used indicates that the effective range for the particular combination of fungus, nutrient and environmental conditions could have been completely overlooked in previous work.

Radial extension and macroscopic appearance of mycelial mats grown in the dark were affected by both auxins and antiauxins, but statistical analysis showed that only on 7 x 10^{-5}M IAA was there a significant increase in growth over that of the controls. Cumulative effects of chemicals were indicated, but in most cases were not proven beyond doubt because there were no significant differences between

radial growth on 14 and 28 x 10^{-5}M concentrations for any of the chemicals used.

Most of the unusual growth patterns (Fig. 4) were stimulated either by MH itself or MH combined with other chemicals. It can not be determined from these data whether these effects are specific or might be elicited by other metabolic inhibitors. Further study will be needed to determine whether the unusual strands stimulated by this chemical (Fig. 4d) differ cytologically from those with less regular branching which are normally produced in the dark. It would also be interesting to know if the more abundant aerial growth on #70A and #70C masks unusual stranding.

Statistical analysis showed that #70A supported growth which differed significantly from that supported by #70C and H_2O except when IAA was combined with MH or when 2,4-D was combined with either MH or TrCA. There were no differences among any of the media when the auxins were combined with MH. Number 70C supported significantly more growth than either #70A or H_2O when 2,4-D was combined with TrCA. EDTA has been shown to produce auxin-like effects in higher plants, but it cannot be determined from the data whether these differences in media were due to increased availability of iron or zinc in #70C or to the EDTA. The above observations raise some questions for further study. Would the same results have been obtained if the inoculum had been grown on #70 without any minor elements? Does this change in minor elements effect responses of illuminated cultures?

The fact that both liquid and agar H_2O-media supported mycelial mats with the same radial area as those supported by #70A and #70C indicates that all necessary nutrients can be translocated from the agar block carrying the inoculum. This translocation did not seem to be affected by the addition of auxins or antiauxins or combinations of these.

While hyphal extension is limited by light and high concentrations of representative auxins, it is not usually limited by diminished moisture supply. When petri dishes were slanted, or for some other reason the liquid medium in part or all of the dish was reduced to a thin layer, the fungus responded with increased stranding and radial growth over the film of liquid (Fig. 3). At times the total area was a factor of 10 or more, greater than that of replications which differed only by the amount of medium in the dish. An extreme example is treatment 23-70A where the one replication with a thin layer of medium had an area of 32 cm^2 and all others had no growth (0.2 cm^2). This phenomenon appeared in all liquid media, but only when the liquid available, in at least part of the dish, was reduced to the point that it would not float the mycelial mat. It was not observed on agar media.

The variation in growth potential of the several pieces of inoculum used has been blamed for at least part of the great variation among replications. Development of a technique to obtain more uniformly viable pieces of inoculum would aid in the evaluation of studies such as these.

If, as has been suggested, the cell walls of this fungus are indeed chitinous, then proof that auxins have a growth regulating role

in fungi similar to that in higher plants would be significant. This knowledge would help elucidate the regulation of growth in fungi, but perhaps just as important, it would provide a key to the mechanism of auxin action in higher plants. It is currently believed that these chemicals act either directly or indirectly on the cell walls. Mediation of the growth of both chitinous and cellulosic walls would indicate that the action is either indirect or that the auxins combine with different co-factors in each case.

SUMMARY

The evidence presented here has not conclusively proven the existence of a role for auxins in the regulation of fungal growth. It has, however, indicated that such a role is possible. The stimulation of radial growth by IAA, the reversible inhibition of growth by white light, and the macroscopically similar appearance of cultures grown in the light and in the presence of auxins in the dark, all show that there may be some link between the action of auxins in higher plants and in fungi.

The observation that illuminated cultures grew in concentrations of 2,4-D which completely inhibited growth in the dark further strengthened this hypothesis. IAA, 2,4-D, TrCA and MH were all shown to be physiologically active in at least some of the concentrations used. Combinations of these chemicals affected growth in a manner which indicated interactions that may have been due to cumulative effects. Statistical variation among replications, probably due in part to variation in inoculum, prevented decisive conclusions on the possible additive effects of combinations of auxins and antiauxins. This approach to the possible role of auxins as fungal growth regulators has not been fully explored and several suggestions for further study have been presented.

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