THESIS

THE RELATION OF FUSARIA TO VARIOUS CULTURE MEDIA

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INTRODUCTION

The selective action of dyes on living cells has been observed for many years. In the last decade, dyes have been much used in media for the isolation and identification of various bacterial organisms. These media have depended partly upon the reaction of a particular organism to the dye, and partly upon the inhibitive properties of the dye toward other organisms. In 1913, the report of Endo's (6) fuchsin-sulphite medium was published. This medium served to differentiate the B. coli group from B. typhosus, the former appearing as red colonies, the latter as clear transparent colonies. Later Holt-Harris and Teague (9) devised their eosin-methylen blue agar for the isolation of B. typhosus. On eosin-methylen blue agar, the B. coli colony has a black center while the B. typhosus colony is clear and transparent. This medium also inhibits the growth of many other water bacteria, and is commonly used in water analysis for a presumptive test. Krumwiede and Pratt (11) successfully isolated typhoid bacilli by using two concentrations of brilliant green which inhibited the growth of other organisms and gave very characteristic colonies of the typhoid bacillus. In 1915, Petrof (14) reported a gentian violet medium for the isolation of the tubercle bacillus, and later Bernstein and Lowe (5) used the same

dye in a medium for the isolation of the influenza organism.

In view of the development of this technique, the question suggested itself,- could dyes be used in differential media for fungi? No reports were found in the literature of any attempts to apply this method for differentiation of fungi in culture. Since such media would assist as much in the task of the identification of fungi as they do in the identification and isolation of bacteria, the problem of testing Fusaria with various dyes was undertaken.

The following experiments were planned and carried out to test the relationship of a fungus to various culture media. The Fusarium group was chosen for the work because it had already been described by Appel and Wollenweber (1), Sherbakoff (16), Link (12), and others whose descriptions and classification are based on measurement, septation, and curvature of the conidia.

Twenty-one water soluble dyes were selected from the several classes of dyes as follows:-

MONAZO	- Chrysoidine Y, Orange G, Ponceau B
DIAZO	- Diamine Blue, Benzopurpurin, Congo Red, Biebrich Scarlet
DIPHENYLMETHANE	- Auromine O
TRIPHENYLMETHANE	- Rosanaline , Malachite Green, Isamine Blue, Crystal Violet, Brilliant Green
XANTHANE	- Eosin (yellow), Rhodamine B
ACRIDINE	- Acridine Yellow

HYDRAZONE - Tartrazine,

QUINONE-IMIDE - Nile Blue A, Magdala Red, Methylene Blue(for bacteria)

This classification is according to Wahl (18) .

Stock solutions of 0.5% concentration of the dyes were made up with distilled water and sterilized by the discontinuous method, in flasks provided with pipettes inserted through the stopper.

PRELIMINARY TESTS

A preliminary test was first undertaken for the purpose of finding a concentration of the dye suitable for the growth of the fungi selected. Fusarium radicicola, F. oxysporum, and F. conglutinans var. callistephi were used in this test.

Method: 10 cc. of a macrospore suspension from a 20 day old culture of the organism were added to a liter flask containing sterile Coon's synthetic medium (5). The Flask was well shaken to secure an even distribution of the spores, and then 10 cc. quantities of the inoculated medium were transferred to sterile preparation dishes by means of sterile pipettes. This procedure was repeated for each organism. The dye solutions were added to the preparation dishes in amounts varying from 1 drop up to 5 drops, making a range of approximate concentrations as follows:- 1 to 40,000 , 1 to 20,000 , 1 to 13,200 , 1 to 10,000 , and 1 to 4,000 . Five preparation dish cultures of each organism were left untreated as checks. The experiment was set up in duplicate. The preparation dishes were placed in clean battery jars, covered with

glass plates, and incubated at room temperature, 20°C., in diffused light. Readings were made at 5, 10, and 20 day intervals. This experiment had the advantage of showing type of growth as well as germination. The results are shown in Table I. No readings are given for benzopurpurin which was found to be precipitated by the medium used, and none are given for auromine which was partially precipitated during sterilization.

This experiment was repeated using the same organisms and method, but omitting tartrazine, acridine, chrysoidine, and orange G. Preparation dishes containing only sterile medium plus dye in the same concentrations as before, were included in this experiments as checks on the color changes which were noted. The results, which are almost identical with those obtained in the first experiment, are recorded in Table II.

These two experiments indicated that the most promising groups of dyes in this work are the triphenylmethane, monazo, and quinone-imide dyes. The other dyes seemed to have no inhibiting effect in the concentrations tried, and show no color changes during the growth of the organisms, although some are able to stain the mycelium.

All the dyes tested were products of the National Analine Co. except Congo red, malachite green, brilliant green, eosin, and crystal violet, which were Grubler's. dyes.

TABLE I

Showing the results of test with dye solutions of various concentration

20 day reading

	F.radicicola						F.congl.cal.						F.oxysporum					
	Growth in					medium decolorized	Growth in					medium decolorized	Growth in					medium decolorized
	1-40,000	1-20,000	1-13,200	1-10,000	1- 4,000		1-40,000	1-20,000	1-13,000	1-10,000	1- 4,000		1-40,000	1-20,000	1-13,200	1-10,000	1- 4,000	
Rosanaline	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Isamine blue	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	—
Malachite green	-	—	-	-	-	—	+	+	+	-	-	+	+	+	-	-	-	+
Brilliant green	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+
Crystal violet	+	+	+	-	-	+	+	-	-	-	-	+	+	+	+	-	-	—
Chrysoidine	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	—
Orange G	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Ponceau	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Diamine blue	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	—
Congo red	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Biebrich scarlet	+	+	+	+	+	—	+	+	+	+	+	-	+	+	+	+	+	—
Rhodamine	+	+	+	+	+	—	+	+	+	+	+	-	+	+	+	+	+	—
Eosin	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Acridine	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Tartrazine	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+	+	+	—
Nile blue	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+	+	+	+
Magdala red	+	+	+	+	+	-	+	+	+	+	+	—	+	+	+	+	+	—
Methylene blue	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+	+	+	—
Checks	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	—

TABLE II

Showing the results of the second test with dye solutions of various concentration

10 day reading

	F.radicicola						F.congl.cul.						F.oxysporum					
	Growth in					medium decolorized	Growth in					medium decolorized	Growth in					medium decolorized
	1-40,000	1-20,000	1-13,200	1-10,000	1- 4,000		1-40,000	1-20,000	1-13,200	1-10,000	1- 4,000		1-40,000	1-20,000	1-13,200	1-10,000	1- 4,000	
Rosanaline	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Isamine blue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Malachite green	+	−	−	−	−	+	+	+	+	−	−	+	+	+	−	−	−	+
Brilliant green	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+
Crystal violet	+	+	+	−	−	+	+	−	−	−	−	+	+	+	+	−	−	−
Ponceau	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Diamine blue	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Congo red	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Rhodamine	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Eosin	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Nile blue	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Magdala red	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Methylene blue	+	+	+	+	+	+	contaminated						+	+	+	+	+	−
Checks	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−

Tests were then made with plate cultures using cheap synthetic medium (5) with the following formula:

cane sugar	7.2 gm.
glucose	3.6
$MgSO_4$	1.23
KH_2PO_4	2.72
KNO_3	2.02
agar	10.0
distilled H_2O	1000

Various amounts of the 0.5 % dye solutions were added, and the resulting colored medium was sterilized in flasks in the autoclave at 15 lbs. pressure for 15 minutes. Heating had no noticable effect on the dyes used.

Method: Plates were poured from the flasks of agar as soon as they came from the autoclave. Pouring the plates while the agar was very hot decreased the chance of contamination. After the plates were cold, they were divided into 2,3 or 4 parts by wax pencil marks on the under side, and numbered. The large number of cultures and media tested made it necessary to use either the divided plate method described by Churchman (4) with several kinds of media in one Petrie dish, or to plant several colonies on the same plate. The latter method was found more satisfactory after some preliminary trials.

Plantings were made from cultures on corn meal or potato dextrose agar. A small, uniform amount of inoculum on a straight needle was used. Aerial mycelium was used where ever possible. In some cases it was necessary to use pionnotes. The plantings were made in a culture room which had previously been steamed and wiped down. All the ordinary sterile precautions were observed and but few contaminations were found in the plates. The

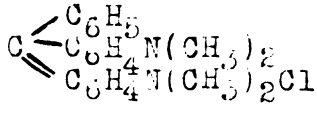
cultures were incubated at approximately 20°C. in a dark oven. Trays of water were placed in the oven to prevent rapid drying out of the cultures. Readings were made at 7, 10, and 14 day intervals. In all experiments, duplicate series were used.

The dyes used in the first experiments were:-

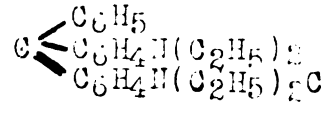
Monazo -	Ponceau, Chrysoidine
Triphenylmethane-	Gentian Violet, Crystal Violet, Malachite Green, Brilliant Green
Quinone-imide-	Nile Blue, Methylene Blue

The organisms used were F. conglutinans, F. radicicola, F. oxysporum, F. batatis, and F. lycopersici.

Cultures on ponceau and chrysoidine media showed no differences from cultures of the same organisms on uncolored cheap synthetic medium, and these dyes were omitted in the later experiments. The organisms tested behaved alike on methylene blue and Nile blue. Methylene blue being the more common, was selected to represent this group of dyes in further investigation. The reactions of these fungi to crystal and gentian violet were similar, crystal violet being slightly more toxic. Gentian violet was chosen for further study because of its common use in bacterial media. Brilliant green was found to be more toxic than malachite green as Klieger (10) demonstrated for bacteria. He explains this on the ground that an ethyl radicle present in the benzene nucleus makes the compound more toxic than does a methyl radicle.

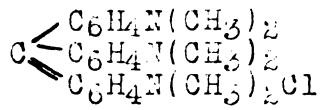


Malachite green

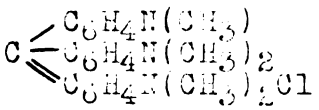


Brilliant green

These tests showed, however, that malachite green is more toxic to the Fusaria tested than gentian violet, the colonies on malachite green medium being ~~being~~ smaller and more slow to develop than those on gentian violet medium of the same concentration*. A greater number of organisms was completely inhibited by malachite green than by gentian violet at the same concentration. This is at variance with the principle of Klieger who states that the greater the number of alkyl radicles present in the benzene nucleus, the more toxic the compound becomes. Gentian violet is a mixture of crysral and methyl violets.



Crystal violet



Methyl violet

He found gentian violet more toxic than malachite green for the bacteria he studied. These results suggest group reactions of the dyes toward the growth of the organisms.

Tests were made using various concentrations of the dyes to find the most suitable concentrations for use in plate cultures. The presence of the agar seemed to exercise a protective action, a greater concentration of the toxic dye being tolerated in a solid medium than in the broth cultures. Tables III and IV show the results of such tests for malachite green and gentian violet. The tests were set up in duplicate on two different occasions.

*Note- These results may misrepresent the true relationships, since they are based on per cent comparisons and not on molecular comparisons.

TABLE III

Comparison of various concentrations of gentian violet, 7 day readings

Name of organism	1 c.c. of 0.5% dye solution per 200 c.c. agar						1.5 c.c. of 0.5% dye solution per 200 c.c. agar						
	diam. more than 2 cm.	diam. less than 2 cm.	submerged	cottony	sericious tomentose	villous	no growth	diam more than 2 cm.	diam. less than 2 cm.	cottony	cottony appressed	sericeous tomentose	submerged
F.conglutinans	–	+	+	–	–	–	–	–	+	–	+ center	–	+
F.batatis	–	+	+	–	–	–	–	–	+	–	–	–	+
F.orthoceras	–	+	+	–	–	⊕	–	–	+	–	–	–	+
F.eumartii	–	+	+	–	–	–	⊕	–	+	+	–	–	–
F.euoxysporum	+	–	–	+	–	–	–	+	–	+	–	–	–
F.aurantiacum	+	–	–	+	–	–	–	+	–	+	–	–	–
F.sclerotioides	+	–	+	–	–	–	–	–	+	–	–	–	+
F.bulbigenum	–	+	–	–	+	–	–	–	+	–	–	⊕	+

TABLE IV

Comparison of various concentrations of malachite green, 7 day reading

Name of organism	0.5 c.c. of o.5% dye solution per 200 c.c. agar							1.5 c.c. of 0.5% dye solution per 200 c.c. agar						3 c.c. of 0.5% dye solution per 200 c.c. agar				
	no growth	diam. more than 2 cm.	diam. less than 2 cm.	submerged	cottony	villous	sericeous tomentose	no growth	diam. more than 2 cm.	diam less than 2 cm.	submerged	cottony	villous	no growth	diam. more than 2 cm.	diam.less than 2 cm.	submerged	cottony
F.conglutinans	–	–	+	+	–	–	–	–	–	+	+	–	+	+	–	–	–	–
F.batatis	–	–	+	+	–	–	+	–	–	+	+	–	–	+	–	–	–	–
F.orthaceras	–	+	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–
F.eumartii	+	–	–	–	–	–	* ⊕	+	–	–	–	–	–	+	–	–	–	–
F.euoxysporum	–	–	+	+	–	–	+	–	–	+	+	center +	–	–	–	+	+	–
F.aurantiacum	–	+	–	–	+	–	–	–	+	–	+	center +	–	+	–	⊕	–	⊕
F.sclerotioides	–	–	+	+	–	+	–	⊕	–	+	+	–	–	+	–	–	–	–
F.bulbigenum	–	–	+	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–

* One tuft of mycelium

The readings marked in circles represent cases where the first and second trials did not give the same results. These contradictions may have been due to varying amounts of inoculum.

FINAL EXPERIMENTS WITH PLATE CULTURES

The media selected for the final tests were made up as follows:-

	Number of c.c. of 0.5% dye solution per 200 c.c. of cheap synthetic agar (page 7)
Malachite green*	1.0 c.c.
Gentian violet	1.5
Methylene blue for Bact.	1.5
Eosin (yellow)	4.0

The method described above was used and all experiments were done in duplicate. All the Fusaria available were grown on these colored media and on uncolored cheap synthetic medium as checks. All the organisms named in the tables were grown in duplicate at three different times, and many of them were grown four times. The results of all the trials were consistant with but slight differences. The organisms tested showed constant characteristics in their behavior toward the dyes used. The source and age of the culture used appeared to be an unimportant factor in the growth on these media as plantings from corn meal and from potato dextrose cultures, and from 2 weeks old and 2 months old cultures of the same organism showed no differences in growth reactions. The following tables are typical of the results obtained. The readings given are the results of the final duplicate series.

*These dyes were Grübler's.

TABLE V

Showing growth on uncolored synthetic medium, 10 day reading

Name of organism	Culture number	Size of colony		Type of colony			Type of mycel.		pigment produced
		Diam. more than 2 cm.	Diam. less than 2 cm.	submerged	center aerial	aerial mycel. abundant	cottony	villous	
F.orthaceras		+	–	+	–	–	–	–	–
F.orth.var.longius		+	–	–	–	+	–	+	–
F.conglutinans		+	–	+	–	–	–	–	–
F.congl.var.cal.		+	–	–	+	–	+	–	–
F.redolens		+	–	–	–	+	+	–	–
F.sclerotioides		+	–	+	–	–	–	–	–
F.oxysporum	209	+	–	–	–	+	+	–	–
"	211	+	–	–	–	+	+	–	–
"	204	+	–	+	–	–	–	–	–
"	160	+	–	+	–	–	–	–	–
"	212	+	–	+	–	–	–	–	–
"	208	+	–	+	–	–	–	–	–
F.euoxysporum		+	–	–	–	+	+	–	–
F.vasinfectum		+	–	–	+	–	+	–	–
F.batatis		+	–	–	+	–	+	–	–
F.tricothecioides		+	–	–	–	+	+	–	–
F.discolor		+	–	+	–	–	–	–	–
F.disc.var.sulph.		+	–	–	+	–	+	–	–
F.culmorum		+	–	–	–	+	+	–	+
F.eumartii	206	+	–	–	–	+	+	–	–
"	171	+	–	–	–	+	+	–	–
F.radicicola	157	+	–	–	–	+	+	–	–
"	202	+	–	–	–	+	+	–	–
"	203	+	–	–	–	+	+	–	–
"	207	+	–	–	–	+	+	–	–
F.solani		+	–	–	–	+	+	–	–
F.coeruleum		–	+	+	–	–	–	–	–
F.zonatum		+	–	+	–	–	–	–	–
F.bulbigenum		+	–	–	+	–	+	–	–
F.aurantiacum		+	–	–	–	+	+	–	–
F.malli		+	–	–	–	+	+	–	–
F.asclerotium	56	+	–	–	+	–	–	+	–
"	213	+	–	–	+	–	–	+	–
"	174	+	–	–	+	–	–	+	–
F.lycopersici	170	+	–	–	+	–	–	+	–
"	153	+	–	–	+	–	–	+	–

TABLE VI

Showing growth on gentian violet medium, 10 day reading

Name of organism	Culture number	Size of colony		Type of colony			Type of mycelium				
		Diam. more than 2 cm.	Diam. less than 2 cm.	submerged	center aerial	aerial mycel. abundant	cottony	villous	sericeous tomentose	medium decolorized	deep purple zone below
F.orthoceras		+	–	–	+	–	–	–	+	–	–
F. " var.long.		+	–	–	–	+	–	–	+	–	–
F.conglutinans	156	+	–	–	–	+	–	–	+	–	–
"	165	+	–	–	–	+	–	–	+	–	–
F. " var.cal.		+	–	–	–	+	–	–	+	–	–
F.redolens		–	+	–	–	+	+	–	–	–	–
F.scleroti.		+	–	–	–	–	–	–	+	–	+
F.oxysporum	209	+	–	–	–	+	+	–	–	–	+
"	211	+	–	–	–	+	+	–	–	–	+
"	204	+	–	–	–	+	–	–	+	–	–
"	160	+	–	–	–	+	–	–	+	–	–
"	212	+	–	–	–	+	–	–	+	–	–
"	208	+	–	–	–	+	–	–	+	–	–
F.euoxysporum		+	–	–	–	+	+	–	–	–	–
F.vasinfectum		+	–	–	–	+	+	–	–	–	–
F.batatis		+	–	–	–	+	–	–	+	–	–
F.tricothec.		–	+	–	–	+	+	–	–	–	–
F.discolor		+	–	–	–	+	–	–	+	–	–
F. " var.sul.		+	–	–	–	+	–	–	+	–	–
F.culmorum		+	–	–	–	+	+	–	–	–	–
F.eumartii	206	–	+	–	–	+	+	–	–	–	–
"	171	–	+	–	–	+	+	–	–	–	–
F.radicicola	157	+	–	–	–	+	+	–	–	+	–
"	202	+	–	–	–	+	+	–	–	–	–
"	203	+	–	–	–	+	+	–	–	–	–
"	207	+	–	–	–	+	+	–	–	+	–
F.solani		+	–	–	–	+	+	–	–	+	–
F.coeruleum		–	+	+	–	–	–	–	–	–	–
F.zonatum		+	–	+	+	–	+	–	–	–	–
F.bulbigenum		–	+	–	–	+	–	–	+	–	–
F.aurantiacum		+	–	–	+	+	+	–	–	+	–
F.malli		+	–	–	–	+	+	–	–	–	–
F.asclerotium	56	+	–	–	–	+	–	–	+	–	–
"	213	+	–	–	–	+	–	–	+	–	–
"	174	+	–	–	–	+	–	–	+	–	–
F.lycopersici	170	+	–	–	–	+	–	–	+	–	+
"	153	+	–	–	–	+	–	–	+	–	+

TABLE VII

Showing growth on malachite green medium, 10 day reading

Name of organism	Culture number	no growth	Size of colony		Type of colony			Type of mycelium		
			Diam. more than 2 cm.	Diam. less than 2 cm.	submerged	center aerial	aerial mycel. abundant	cottony	villous	sericeous tomentose
F.orthaceras		–	–	+	+	–	–	–	–	–
F.orth.var.longius		–	–	+	–	–	+	+	–	–
F.conglutinans	156	–	+	–	–	–	+	+	–	–
"	165	–	+	–	–	–	+	+	–	–
F. " var.cal.		–	+	–	–	+	–	+	–	–
F.redolens		–	+	–	–	–	+	–	–	+
F.sclerotioides		–	–	+	–	+	–	–	–	+
F.oxysporum	209	–	+	–	+	–	–	–	–	–
"	211	–	+	–	+	–	–	–	–	–
"	204	–	+	–	–	–	+	–	–	+
"	160	–	+	–	–	–	+	–	–	+
"	212	–	+	–	–	–	+	–	–	+
"	208	–	+	–	–	–	+	–	–	+
F.euoxysporum		–	+	–	–	–	+	+	–	–
F.vasinfectum		–	+	–	–	–	+	+	–	–
F.batatis		–	–	+	+	–	–	–	–	–
F.tricothecioides		+	–	–	–	–	–	–	–	–
F.discolor		+	–	–	–	–	–	–	–	–
F. " var.sulph.		–	+	–	–	+	–	–	+	–
F.culmorum		+	–	–	–	–	–	–	–	–
F.eumartii	206	+	–	–	–	–	–	–	–	–
"	171	+	–	–	–	–	–	–	–	–
F.radicicola	157	–	+	–	–	–	+	+	–	–
"	202	–	+	–	–	–	+	+	–	–
"	203	–	+	–	–	–	+	+	–	–
"	207	–	+	–	–	–	+	+	–	–
F.solani		–	+	–	–	–	+	–	–	+
F.coeruleum		+	–	–	–	–	–	–	–	–
F.zonatum		–	+	–	–	–	+	+	–	–
F.bulbigenum		+	–	–	–	–	–	–	–	–
F.aurantiacum		–	+	–	–	–	+	+	–	–
F.malli		–	–	+	–	–	+	+	–	–
F.asclerotium	56	–	+	–	–	–	+	–	–	+
"	213	–	+	–	–	–	+	–	–	+
"	207	–	+	–	–	–	+	–	–	+
F.lycopersici	170	–	–	+	–	+	–	–	–	+
"	153	–	–	+	–	+	–	–	–	+

TABLE VIII

Showing growth on eosin medium,
10 day reading

Name of organism	Culture number	Size of colony		Type of colony			Type of mycelium				zonation
		Diam. more than 2 cm.	Diam. less than 2 cm.	submerged	center aerial	aerial mycel. abundant	cottony	villous	cottony appressed	sericeous tomentose	
F.orthaceras		+	–	–	–	+	–	–	+	–	–
F. " var.long.		+	–	+	–	–	–	–	–	–	–
F.conglutinans	156	+	–	+	–	–	–	–	–	–	–
"	165	+	–	+	–	–	–	–	–	–	–
F. " var.cal.		+	–	–	–	+	–	+	+	–	–
F.redolens		+	–	–	–	+	+	–	–	–	–
F.sclerot.		+	–	–	+	–	–	–	–	+	+
F.oxysporum	209	+	–	–	–	+	+	–	–	–	–
"	211	+	–	–	–	+	+	–	–	–	–
"	204	+	–	–	+	–	–	+	–	–	–
"	160	+	–	–	+	–	–	+	–	–	–
"	212	+	–	–	+	–	–	+	–	–	–
"	208	+	–	–	+	–	–	+	–	–	–
F.euoxysporum		+	–	–	–	+	+	–	–	–	+
F.vasinfectum		+	–	–	–	+	+	–	–	–	–
F.batatis		+	–	–	+	–	+	–	–	–	–
F.tricothec.		+	–	–	+	–	–	–	–	+	–
F.discolor		+	–	–	–	+	+	–	–	–	–
F. " var.sul.		+	–	–	+	–	–	–	+	–	–
F.culmorum		+	–	–	–	+	+	–	–	–	–
F.eumartii	206	+	–	–	+	–	+	–	–	–	–
"	171	+	–	–	+	–	+	–	–	–	–
F.radicicola	157	+	–	–	–	+	+	–	–	–	–
"	202	+	–	–	–	+	+	–	–	–	–
"	203	+	–	–	–	+	+	–	–	–	–
"	207	+	–	–	–	+	+	–	–	–	–
F.solani		+	–	–	–	+	+	–	–	–	–
F.coeruleum		–	+	–	–	–	–	–	+	–	–
F.zonatum		+	–	+	–	–	–	–	–	–	–
F.bulbigenum		+	–	–	–	–	–	–	+	–	–
F.aurantiacum		+	–	–	–	+	+	–	–	–	–
F.malli		+	–	–	–	+	+	–	–	–	–
F.asclerotium	56	+	–	–	–	+	–	+	–	–	–
"	213	+	–	–	–	+	–	+	–	–	–
"	174	+	–	–	–	+	–	+	–	–	–
F.lycopersici	170	+	–	–	–	+	–	–	+	–	–
"	153	+	–	–	–	+	–	–	+	–	–

TABLE IX

Showing growth on methylene blue medium, 10 day reading

Name of organism	Culture number	Size of colony		Type of colony			Type of mycelium			medium becomming green below	medium becomming lavender spotted
		Diam. more than 2 cm.	Diam. less than 2 cm.	submerged	center aerial	aerial mycel. abundant	cottony	villous	sericeous tomentose		
F.orthaceras		+	-	+	-	-	-	-	-	-	+
F. " var.long.		+	-	-	-	+	+	-	-	-	+
F.conglutinans	156	+	-	-	+	-	-	-	+	-	+
"	165	+	-	-	+	-	-	-	+	-	+
F. " var.cal.		+	-	-	+	-	+	-	-	-	+
F.redolens		+	-	-	+	-	-	-	+	-	+
F.sclerot.		+	-	-	+	-	-	-	+	-	+
F.oxysporum	209	+	-	-	-	-	-	-	-	-	-
"	211	+	-	-	-	-	-	-	-	-	-
"	204	+	-	-	-	-	-	-	-	-	-
"	160	+	-	-	-	-	-	-	-	-	-
"	212	+	-	-	-	-	-	-	-	-	-
"	208	+	-	-	-	-	-	-	-	-	-
F.euoxysporum		+	-	-	-	-	-	-	-	-	-
F.vasinfectum		+	-	-	-	+	+	-	-	-	+
F.batatis		+	-	-	-	-	-	-	-	-	-
F.tricothec.		+	-	-	-	-	-	-	-	-	-
F.discolor		+	-	-	-	-	-	-	+	-	+
F. " var.sul.		+	-	+	-	-	-	-	-	+	+
F.culmorum		+	-	+	-	-	-	-	-	-	+
F.eumartii	206	+	-	-	-	+	+	-	-	-	+
"	171	+	-	-	-	+	+	-	-	-	+
F.radicicola	157	+	-	-	-	+	+	-	-	+	-
"	202	+	-	-	-	+	+	-	-	+	-
"	203	+	-	-	-	+	+	-	-	+	-
"	207	+	-	-	-	+	+	-	-	+	-
F.solani		+	-	-	-	+	+	-	-	-	-
F.coeruleum		-	+	+	-	-	-	-	-	-	-
F.zonatum		+	-	-	+	-	-	-	+	-	+
F.bulbiginum		+	-	-	-	-	-	-	+	-	+
F.aurantiacum		+	-	-	-	+	+	-	-	-	+
F.malli		+	-	-	-	+	+	-	-	-	-
F.asclerotium	56	+	-	+	-	-	-	-	-	-	+
"	213	+	-	+	-	-	-	-	-	-	+
"	174	+	-	+	-	-	-	-	-	-	+
F.lycopersici	170	+	-	+	-	-	-	-	-	-	+
"	153	+	-	+	-	-	-	-	-	-	+

The accompanying photographs show the types of colonies described. There are three distinct types. The term cottony is used to describe the most common type. The mycelial threads are fine and soft, not matted together but intermingled like the fibers of cotton. Cottony appressed designates a similar type of mycelium , but less dense, and closer to the surface of the medium. The villous colony is covered with fine, soft, short hair, not matted together, and not very dense. In the colony described as sericious tomentose, the mycelial threads are long, soft,and silky, several threads being stuck together to form the long silky tufts as shown in the photograph of F. solani . F.culmorum and F.tricothecioides are classed as cottony because their mycelium is of that type, but it is loose and spreading like a weft of cotton pulled out very thin. The growth of F. culmorum usually spreads to the top of the Petri dish and is torn in removing the cover. No good pictures were obtained for this reason. F. culmorum produced its typical red color even when grown in the dark. None of the other Fusaria tested produced any color on untreated synthetic medium under these conditions. In many cases only the center portion of the colony produced any aerial mycelium. Such colonies are classified in the tables under the heading "center aerial," while the heading "aerial mycelium abundant" is used to designate those colonies which were covered over the entire surface with aerial mycelium. F.sclerotioides differed from all the other colonied on eosin and gentian violet in having a colony entirely submerged save for a zone

of sericious tomentose aerial mycelium near the edge of the colony.

The color changes in medium recorded apoear on the under side of the plate, and are very marked. On gentian violet, F.malli, F.solani, and two of the cultures of F.radicicola decolorize the medium so that not the slightest trace of color remains, and the medium appears white against the white mycelium above. The colonies of the other organisms appear distinctly lavender below. Certain organisms as F.oxysporum show a deep purple ring near the edge of the colony. This ring is much deeper in color than any of the rest of the medium. The colored zone recorded for F.euoxysporum on eosin appears in the mycelium which is white save for a narrow pink zone half way to the edge of the colony, where the dye has been absorbed by the mycelium. F.aurantiacum changes eosin from its normal yellowish pink to a pure pink. This is observed on the lower side of the plate. The lavender spotting on the methylene blue plates is due to the alkalinity produced by the fungi. The characteristics of growth selected and recorded in tables 5 to 9, are definite and constant under the conditions of the experiment.

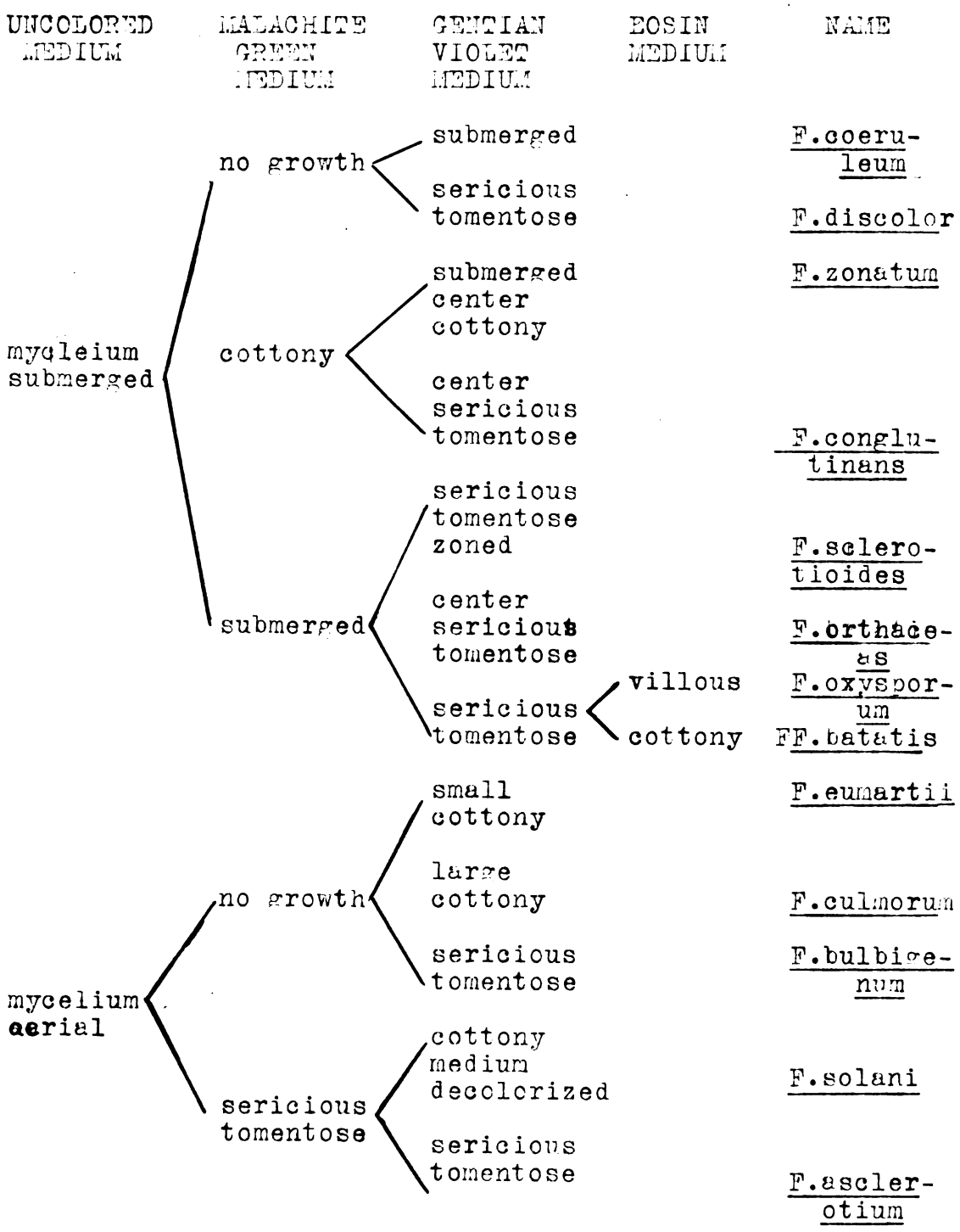
The four cultures of F. radicicola tested were from entirely different isolations, and identified by different authorities; the growth on the media used was identical in every respect save one. Although the appearance of the mycelium was the same on gentian violet medium, two of the cultures decolorized the dye, while the

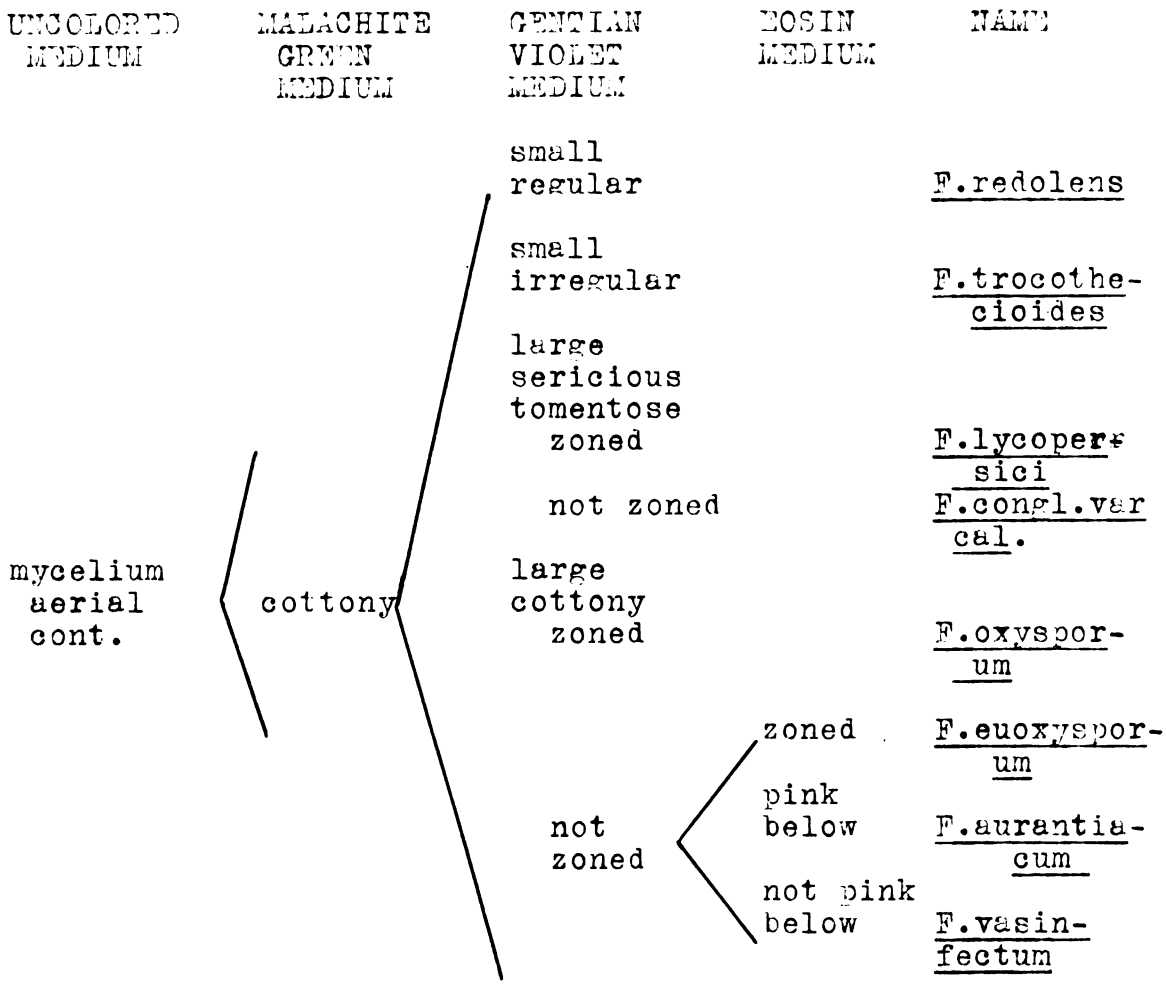
other two did not. F. lycopersici 170 and 153 were from different isolations, and grew identically alike in every respect. The histories of cultures 171 and 206 of F.eumartii show them to be sub-cultures of the same isolation, although they were obtained from different laboratories. Their growth was identical. Three separate isolations of F. asclerotium produced cultures which grew alike on these media. Of the six different cultures of F.oxysporum tried, two strains appear on the media used. Cultures 209 and 211 are alike but quite distinct from the other four cultures. The two cultures of F.conglutinans are from separate isolations, and produce the same type of colony.

The results show that the cultural characteristics of these Fusaria are constant under a given set of conditions. As pointed out by Appel and Wollenweber, the type of colony whether submerged or aerial, depends somewhat upon moisture. Moore (13) also shows that the production of aerial mycelium depends upon the H ion contration as well as the sugar concentration of the medium. However, under constant conditions, the cultural characteristics seem to remain constant.

The following schematic outline was devised to test the usability of these cultural characters as a basis for identification. No work was done in this investigation on identifying the organisms according to spore measurements. Only such cultures were used as had been identified previously by an authority on Fusaria, but all of the cultures had been transfered several times,

and no responsibility is assumed for the correct naming of the cultures used. It was not found necessary to use the characteristics on all the media to distinguish between these fungi. Other classifications could be devised using other characters. The following outline is given to illustrate the possibilities in this regard.





The behavior recorded in this paper is based on the reactions of cultures merely transfered from laboratory media without any attempt to produce the condition refered to as "hoch kulture".

ENZYME EXPERIMENTS

Studies were undertaken to discover the cause of the color changes and decolorization of the dyes used. Since the triphenylmethane dyes had already been found to be toxic to Fusaria, the plan was adopted of growing the organism several weeks in uncolored synthetic broth, and then adding a high concentration of dye after abundant growth was obtained.

Flasks containing sterile Coons'synthetic broth were inoculated with F.radicicola and F.conglutinans

and grown at room temperature in diffused light for three weeks. A thick mat of mycelium had formed at that time. 1 c.c. of malachite green was added to certain of the flasks, the others being held as checks. The flasks were incubated at room temperature over night. All the flasks treated with dye were completely decolorized. 0.5 c.c. of the dye was added to each flask previously treated. The dye was decolorized in three hours. One of these flasks containing a 25 day old culture of F.radicicola which had decolorized malachite green was used in the following experiment:

The mat of mycelium was filtered off on a Buchner funnel. The clear culture fluid left was designated as the"filtrate" The mycelium was washed to remove traces of the filtrate,and ground up in a mortar with sterile distilled water and quartz sand. The liquid was filtered off and refiltered through sterile filters until clear. This liquid was designated as the "extract". Both filtate and extract were divided into two portions, one part being boiled for 5 minutes and cooled. A slight precipitate formed on boiling. Sterile tubes containing 10 c.c. lots of filtrate and extract, boiled and unboiled, were set up, and 1 drop of 0.5 % solution of malachite green added to each tube. Tubes containing the same amounts of sterile distilled water and dye were used as checks. The tubes were incubated at 25°C. for 12 hours and read. The results of this experiment are given in table X.

TABLE X

Showing the effect of heat on mycelial extract and culture fluid in their reactions toward malachite green

filtrate boiled	--	color unchanged
filtrate unboiled	--	decolorized
extract boiled	--	color slightly paler
extract unboiled	--	decolorized
checks	--	color unchanged

This result pointed toward an enzyme reaction. There was a possibility that the decolorization might be due to the presence of ammonia which could decolorize malachite green by precipitating the carbinol form of the dye, or might produce sufficient alkalinity to allow the aldehyde sugar to reduce the dye as Gates and Olitsky (7) demonstrated. The NH_3 would be removed by boiling which would explain the results above. It was therefore necessary to determine if NH_3 was the factor.

A two weeks old culture of F.conglutinans on Coons synthetic broth in which the asparagin was replaced by KNO_3 to avoid the NH_2 groups, was tested for its ability to decolorize malachite green. It was found to decolorize rapidly. The pH value was determined by the colorimetric method described by Clark (3) to be 6.5. A standard buffer solution with a pH value of 6.5 was made up and 10 c.c. portions tested with 1 drop each of malachite green. The tubes were incubated at 25°C. for 24 hours.

Results:

buffer solution pH 6.5	color unchanged
culture fluid pH 6.5	decolorized
buffer solution pH 6.5 with dextrose	unchanged
distilled H_2O checks	unchanged

These results indicate that the H ion concentration is not the significant factor and that some other agency

than NH_3 was responsible for the decolorization. Tests were then started to see if the decolorization was due to enzymic action.

PRECIPITATION TEST

A ten day old culture of F.conglutinans var.cal. grown on cheap synthetic broth was tested for ability to decolorize malachite green. It decolorized 10 drops of 0.5% malachite green in one hour. The mycelial mat was filtered off, and 100 c.c. of the clear culture fluid treated with enough 97% alcohol to make an 80% alcoholic solution. A flocculent precipitate was formed. This was allowed to settle, and the supernatent liquid was siphoned off. The precipitate was dried at room temperature, and ground to a powder. A yield of 250 mg. was obtained. 100 mg. of the powder were redissolved in 100 c.c. of sterile distilled water. All the material did not dissolve on 15 minutes standing. The solution was divided into two equal lots, one half being filtered through sterile filter paper. Both the filtered and unfiltered solutions were further divided into equal portions , one half being boiled for 10 minutes. A precipitate was formed on boiling. 5 c.c. portions of the solutions thus prepared were placed in sterile tubes by means of sterile pipettes, and 1 drop of a 0.5% dye solution was added to each tube. The test was set up in duplicate. After 12 hours incubation at 25°C. the results were read as follows:

	Malachite green	Gentian violet	Methylene blue
unfiltered unboiled	decolorized	partly decolorized	partly decolorized
unfiltered boiled	unchanged	unchanged	unchanged
filtered unboiled	decolorized	partly decolorized	partly decolorized
filtered boiled	unchanged	unchanged	unchanged
distilled water checks	unchanged	unchanged	unchanged

The agent responsible for the decolorization was found to be precipitated by 80% alcohol, not removed by filtration, but was destroyed by heating.

It is well known that the triphenylmethane dyes may be decolorized by oxidation or reduction, while methylene blue is decolorized by reduction. Flask cultures of these organisms treated with methylene blue had several times been observed to have a colerless layer at the bottom, while the top layer remained blue. This suggested the influence of atmosphereic oxygen. Several flasks of modified Coons synthetic solution were inoculated with F.radicicola and F. conglutinans. After growth had progressed for 10 days, dye solutions were added and part of the cultures were sealed with parafin melted and cooled to about 50° C. These cultures were incubated at 25°C. After one hour all the sealed cultures had completely decolorized while the unsealed cultures were only slightly paler. The seal was broken on the cultures and the contents of the flasks shaken up.

Methylene blue regained its color almost immediately on contact with the air, but malachite green, gentian violet, ponceau, and chrysoidine remained pale.

100 mg. of the alcoholic precipitate described above were dissolved in 100 c.c. of sterile distilled water and a test with dyes was set up as before. The tubes were sealed with parafin and incubated as before. All three colors were completely decolorized in the unboiled tubes. These results indicate that decolorization is due to enzymic action, and is probably a reduction. It is a matter of contention whether living tissues secrete reductases capable of reducing dyes to their leucobases as claimed by Ricketts (15) and Harris (8) or whether such decolorization is due to the SH groups in proteins and amino acids as cystein. Heffter, Strassner (17), and others maintain the latter view. As the evidence against the existance of such reductases is not generally accepted, and since so little is known of the nature of fungus proteins, it seems that the results of these tests are most simply explained by attributing the decolorization to a reducing enzym of the types already found in organic material.

SUMMARY

The growth of various Fusaria was tested on twenty different dyes. In their effects upon these organisms, groups or classes of dyes seemed to show reactions. The triphenylmethane dyes are the most toxic to the fungi tested. Some of these organisms are able to decolorize the triphenylmethane, monazo, and quinone-imide dyes

tested. The agent of decolorization is believed to be enzymic in nature, and the reaction is probably a reduction since it takes best in the absence of air.

Under the conditions of the experiments, the fungi tested showed definite and consistant characteristics of growth on synthetic media both colored and uncolored. The previous cultural conditions of the fungus do not seem an important factor in the growth on synthetic media.

The results indicate that synthetic media and synthetic media colored by dyes may furnish valuable means for quick and accurate identification of certain groups of fungi difficult of identification by the ordinary morphological methods.

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PLATE I

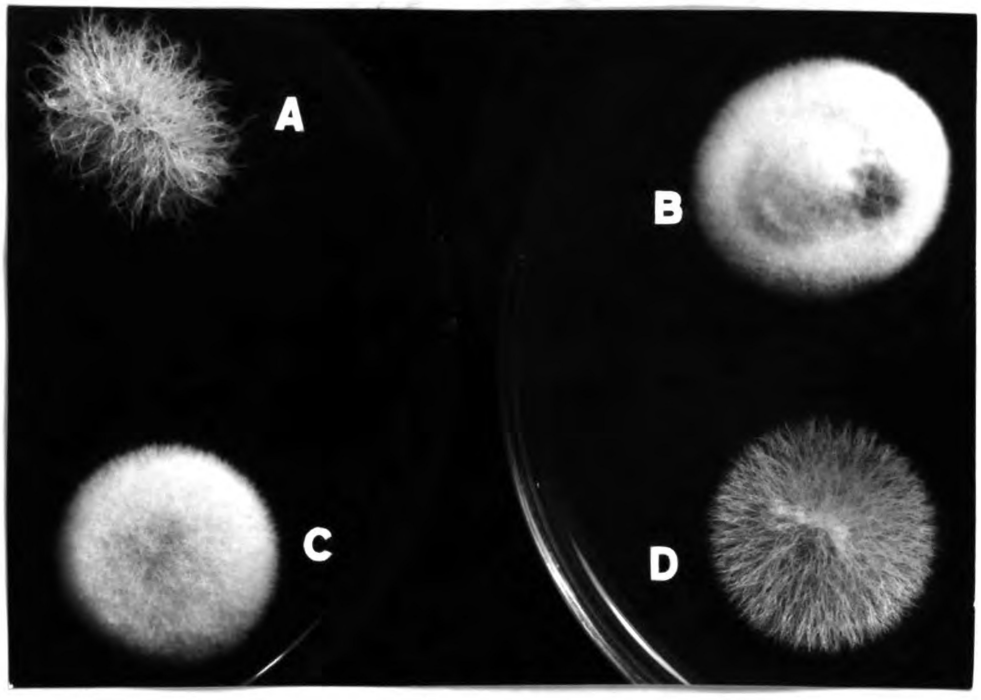

Fig.1.
A- Sericeous tomentose type of colony (F.solani)
B- Cottony type of colony (F.vasinfectum)
C- Cottony type of colony (F.zonatum)
D- Villous type of colony (F.discolor sulph.)
on malachite green medium

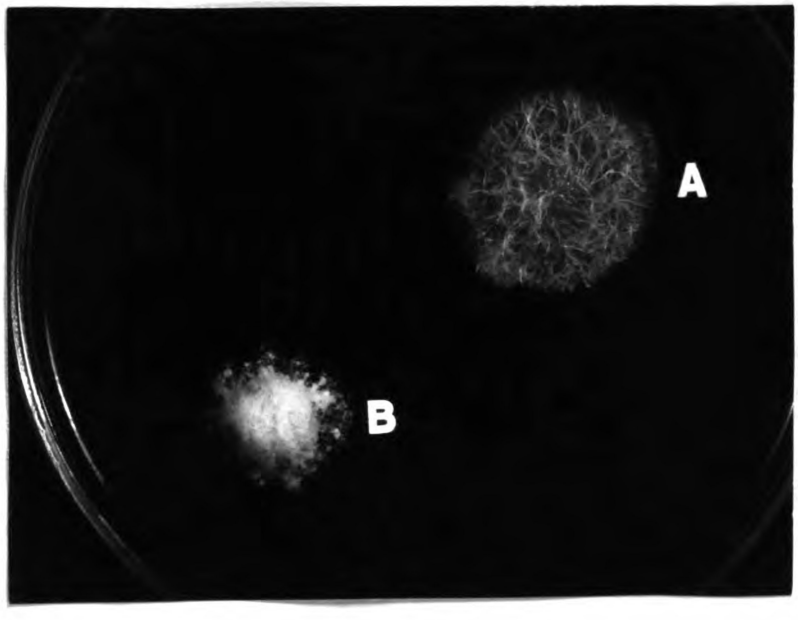

Fig.2.
A- F.conglutinans on gentian violet medium
B- F.tricothecioides on same medium

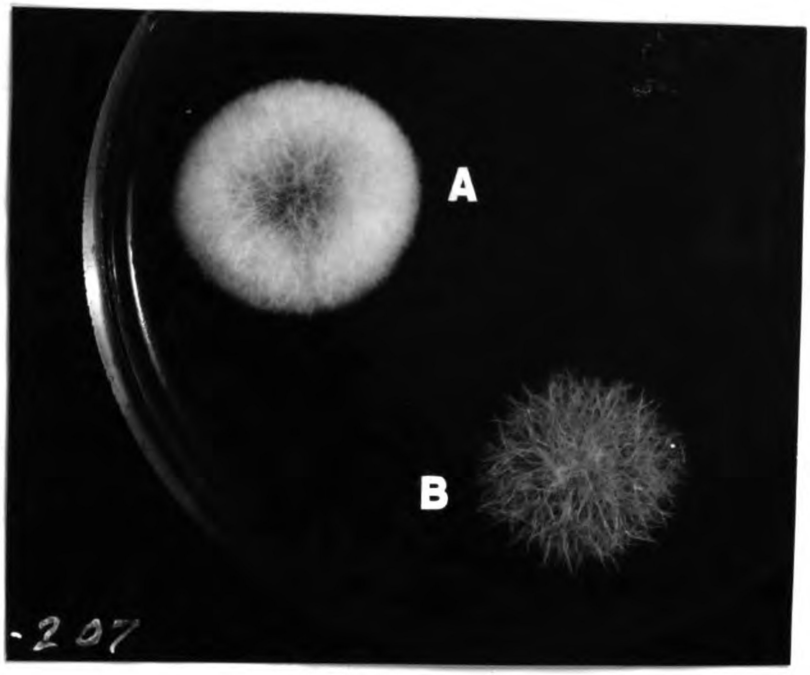

Fig.1.
A- F.orthaceras longius on gentian violet
B- F.lycopersici on same medium

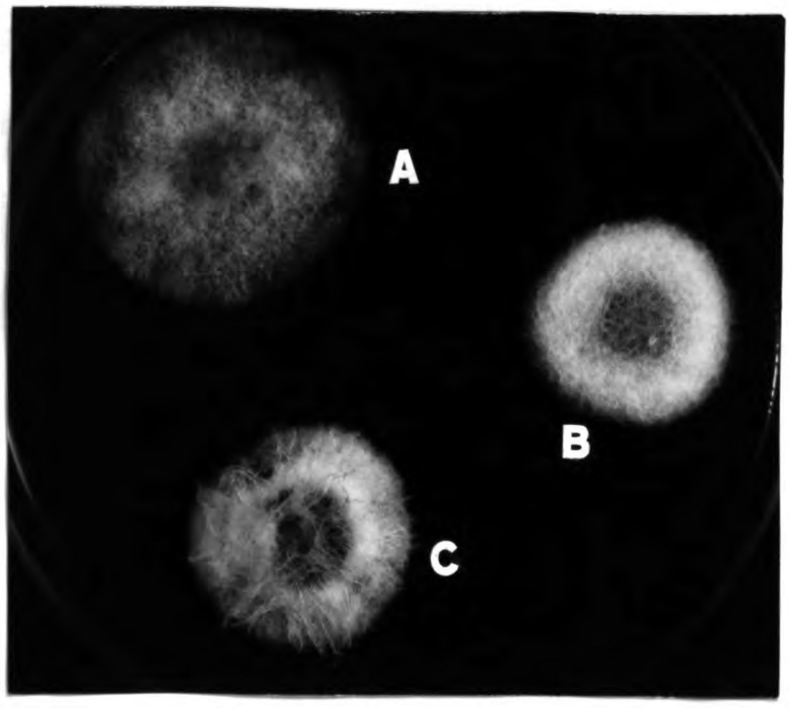

Fig.2.
A- F.radicicola on malachite green
B- F.oxysporum 211 on the same medium
C- F.oxysporum 204

PLATE III

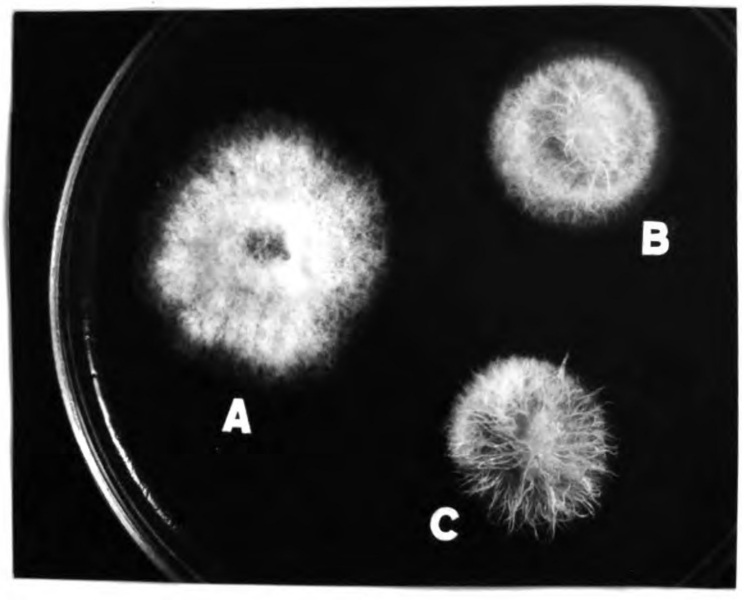

Fig.1
A- F.radicicola on gentian violet
B- F.oxysporum 211 on same medium
C- F.oxysporum 204

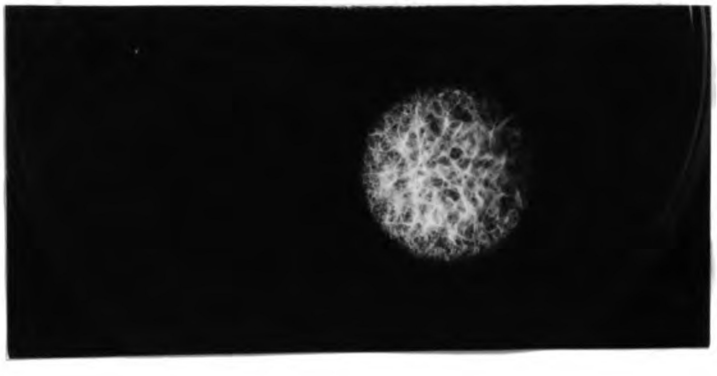

Fig.2
F.conglutinans on malachite green