Serological study of Ustilago Zeae and other basidiomycetes
by George F Parke

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Botany and Bacteriology at Montana State College
Montana State University

Abstract:
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SEROLOGICAL STUDY OF USTILAGO ZEAE AND OTHER BASIDIOMYCETES

INTRODUCTION

Originally the reactions of immunity were studied with the purpose of solving urgent problems concerning the cure, diagnosis and prevention of disease. Since that time there has come to be a growing recognition of its importance in determining the relationship of specific proteins in various plants and animals. Its importance has been emphasized by Mez and his co-workers (17) who have made the most extensive series of investigations on the serological reactions of specific plant proteins, and have set up a phylogenetic classification of plants based on serological reactions of their proteins. Link (13) has applied the precipitin ring test to "Fungi II", and was able to differentiate two strains of Sclerotinia fructicola as well as the plus and minus strains of Neurospora tetrasperma. Martin and Cotner (15) found some phylogenetic significance in their serological work on the moth protein. Keston (12) working with fungi pathogenic to man, used as test antigens water soluble polysaccharide fractions and reported very high titers. Matsumoto (16) found that compliment fixation tests were more satisfactory than either precipitin or agglutination tests for the identification of Aspergillis. He also concluded that it was possible to differentiate the genera of the fungi; but his

results as to species were not clear enough to indicate a differentiation between them. L. Almond and Stovall (1), working with Monilia, inoculated the experimental animals with the living cells and following the third injection received a titer of nearly 1-400. The inoculations were given intravenously at two day intervals; the first inoculation included 150,000,000 to 250,000,000 organisms with increased doses for the following injections. In this work they were not able to demonstrate satisfactorily by serological means the different species of Monilia studied.

The present study was primarily undertaken to determine by serological methods some of the physiologic strains of Ustilago zeae (Beckm.) Ung. Later it was extended to certain fleshy basidiomycetes, namely, three species of Hydnum, two species of Coprinus, one of Psalliota and one of Pleurotis.

It has been suggested by some earlier authors that the strains of Ustilago zeae are only phenotypic and that the forms change readily by a process of adaptation. If this is true the possibility of distinguishing the strains serologically are small. Stakman (19), from whom a great part of the methods of culturing the smut has been taken, disproves the above statement to a considerable extent, and maintains that the new physiologic forms originate by hybridization and mutation.

MATERIALS AND METHODS

The fungi studied are: Ustilago zeae (Beckm.) Ung., Hydnum coralloides Scop., Hydnum coput-ursi Fr., Hydnum septentrionale Fr.- Northern, Pleurotus ostreatus Jacq., Coprinus atromentarius (Bull) Fr., Coprinus comatus Fr., Psalliota campestris Linn. The strains of Ustilago zeae were furnished by J. J. Christensen of the University Farm, St. Paul, Minnesota. The species of Hydnum, Coprinus, Psalliota, and Pleurotis were taken from Dr. F. B. Cotner's collection of fungi at Montana State College.

In an attempt to prepare the smut spores in such a condition so as to make it suitable for intraperitoneal injections difficulties arose. In solving these difficulties some preliminary work was carried out with Ustilago zeae in 1934 during which time the author attempted to grind the spores fine enough for intraperitoneal injections. This method failed because of the tendency for the spores to adhere to one another and consequently clog the syringe. However, later a scheme was devised which involved the culturing of the U. zeae on artificial media.

For the purpose of culturing the smut, potato dextrose agar suggested by Stakman and Christensen (19) was selected because of the ease with which it was prepared, and because of the abundant growth that was secured. It did not seem advisable to attempt to grow the cultures in a protein-free nutrient solution even though this procedure would avoid introducing foreign proteins into the experiment,

because the method used seemed efficient, and left little room for error. To obtain pure culture growths 0.1 g. of chlamydospores was allowed to soak in 100 cc. of a one per cent copper sulphate solution for twenty to thirty hours. The spores were then removed to sterile centrifuge tubes where they were washed twice with sterile distilled water and then transfered to a flask containing 100 cc. of sterile water in which they were allowed to stand for another 20 to 30 hour period. They were taken from this water by means of a sterile capillary tube and sprayed on to the surface of the potato dextrose agar plates held at room temperature. Germination took place within two or three days and the germinating spores were transferred by means of a platinum loop to potato agar slants.

To obtain two or three grams of the dried protein of each species, for inoculation, it was necessary to cover the surface of the agar slants in Erlenmyer flasks with the growth from the germinated spores. In the course of from five to seven days a maximum growth appeared which was carefully removed and placed on a watch glass and dessicated at 55° C. It required, on the average, ten 250 cc. Erlenmyer flasks containing 75 cc. of the agar to secure a sufficient growth to make a single series of inoculations.

Growth of the Organisms.- All strains of the smut grew readily after the initial germination had taken place. However, in a majority of the cases they differed decidedly from each other in culture characters, including rates of growth, consistency of the colony, surface characters,

and color. An idea of the differences in general cultural characters can be gained from Table I, in which are listed some of the characteristics on potato dextrose agar. The numbers given for the different strains were just taken arbitrarily by the author to distinguish the different samples used.

Table II

Cultural characters of 7 lines of U. zeae growing on potato dextrose agar

Strain No.	Color	Elevation	Luster	Topography	Margin
1.	Orange brown covered with white powder	Convex	Dull	Verrucose	Fimbriate
2.	Pinkish buff	Convex	Dull waxey	Rugose	Finely lobate
3.	Orange brown covered with white powder	Convex	Dull	Coarsely Verrucose	Undulate
4.	Purple brown	Convex	Dull waxy	Rugose	Somewhat lobate
5.	Orange buff	Convex	Dull waxy	Coarsely Rugose	Lobate
6.	Brown grey	Convex	Dull	Coarsely Verrucose	Lobate
7.	Purple brown covered with white powder	Convex	Dull	Rugose	Entire

Although the different strains varied somewhat as to their texture, all were viscous enough to facilitate the harvesting of the cultures. The growth characteristics of the organisms used in this work as shown in Table I, agree closely with those recorded by Stakman (19).

Immunization procedure similar to that developed by Almond (1) was attempted by the author in his preliminary work with the U. zeae,

but met with no success.

However, the antiserum was successfully prepared by inoculating rabbits intraperitonealy at 5 day intervals with a weighed amount of the ground, dried culture suspended in 5 cc. of sterile physiological salt solution. Six such injections were made in each animal with gradual increasing amounts of the dried protein. After the first inoculation all of the experimental animals suffered a severe shock, resulting in loss of appetite amd loss of weight. All of the rabbits recovered with the exception of two which were replaced during the second series of inoculations. In the injections following the first the rabbits were noticeably affected for approximately a day after inoculation but invariably recovered to normal on the second day. It was not apparent that any one protein stimulated a greater reaction than that of any other. A post mortem held on the two dead rabbits showed evidence that their death was due to the suspended protein being accidentally injected into the intestine, thus causing an anaphylactic shock. Other than the two fatalities mentioned above, no interruption occurred in the immunization of the experimental animals.

The details of the rabbit immunizations are given in Table II.

Table II

Organisms used in inoculation	All inoculations were made at 5 day intervals					
Species	1st	2nd	3d	4th	5th	6th
Coprinus atrementarius	150 mg. of dried protein suspended in 5 cc. of 0.85% NaCl	200 mg. suspended in 5 cc. of 0.85% NaCl	200 mg. suspended in 5 cc. of 0.85% NaCl	250 mg. suspended in 5 cc. of 0.85% NaCl	300 mg. suspended in 5 cc. of 0.85% NaCl	300 mg. suspended in 5 cc. of 0.85% NaCl
Coprinus comatus						
Hydnum septentrionale						
Hydnum coralloides						
Ustilago zeae 1						
Ustilago zeae 2						
Ustilago zeae 3						
Ustilago zeae 4						
Ustilago zeae 5						
Ustilago zeae 6						
Ustilago zeae 7						

Following the fifth inoculation blood was withdrawn from the marginal ear vein for the purpose of running preliminary tests to determine the strength of the titer developed. All of the rabbits were found to have developed immunity for the homologous antigen, but the titer was not sufficiently high, therefore another injection was administered. Six days after the sixth injection, the 12 rabbits were bled aseptically from the heart. The sample of blood was placed in sterile centrifuge tubes and allowed to clot, they were then taken into

the refrigerator for thirty minutes to hasten separation of the serum. The serum obtained by centrifuging was pipetted into sterile ampules which were sealed and placed in the refrigerator for future use.

The antigens to be used in the tests were prepared by placing 100 mg. of the ground protein and 3 cc. of physiological saline in weighed centrifuge tubes. These were kept in the refrigerator for 24 hours (shaken frequently); they were then centrifuged and as much as possible of the supernatant liquid pipetted into tubes. The residue was thoroughly dried and weighed to determine the loss in weight which was considered as the weight of the material extracted. Dilutions were made from the supernatant liquid with corrections for the weight of the sodium chloride.

EXPERIMENTAL DATA

Glass tubing with an inside diameter of 4 mm. cut in 6 to 7 cm. lengths was sealed off and used for the precipitin tests. A column of serum nearly .5 cm. long was placed in the bottom of the test tube; and approximately the same volume of the diluted extract was added to the serum. This was done by the use of a capillary tube, care being taken that a precise line remained between the two liquids.

The method used in recording the reactions is taken from Martin & Cotner (15). A profuse precipitate showing considerable depth at the junction of the antiserum and the extract was read as a four-plus (++++), a ring with less depth was read as a three-plus (+++), the

lighter but definite rings as two-plus reactions (++), and a reaction in which a definite ring was not formed but in which a slight precipitate was perceptible was read as a one-plus (+) reaction. Checks were made on the various reagents preceding each setup to make sure that the test material had not altered from time to time.

Table III shows the results of the reaction of the antigens with their homologous antiserum.

Table III

Precipitin Reaction
Titers of Homologous Serums and Antigens

Species	Antigen Dilutions						
	1-400	1-800	1-1600	1-2400	1-3200	1-4800	1-6400
Coprinus atramentarius	#++++	+++	++	+	+	+-	-
	++++	++++	+++	+++	++	+	-
Coprinus comatus	++++	+++	++	+	+	+-	-
	++++	++++	+++	++	++	+	-
Hydnum septentrionale	++++	+++	++	++	+	+-	-
	++++	++++	+++	++	++	+	-
Hydnum coralloides	++++	+++	++	+	+	-	-
	++++	++++	+++	++	+	+	-
Ustilago zeae No. 1	+++	++	+	+	-	-	
	++++	+++	++	+	-	-	
Ustilago zeae No. 2	+++	++	++	+-	-	-	
	++++	+++	++	+	-	-	
Ustilago zeae No. 3	+++	++	+	+-	-	-	
	++++	+++	++	+	-	-	
Ustilago zeae No. 4	+++	+++	++	+	-	-	
	++++	+++	+++	++	+	-	
Ustilago zeae No. 5	+++	++	+	+-	-	-	
	++++	+++	++	+	-	-	
Ustilago zeae No. 6	+++	++	++	+	+-	-	
	++++	+++	+++	++	+	-	
Ustilago zeae No. 7	+++	++	++	+-	-	-	-
	+++	+++	++	+	-	-	-

#Signs in upper part of space are readings for 1st hour, lower part for 2nd hour.

It will be noticed that all of tests, when the homologous antiserum was used, produced a reaction at a dilution not lower than 1-2400. The proteins from the strains of Ustilago, which were ground equally as fine as the other proteins, did not produce antibodies in the experimental animal that reacted at as high a dilution as did the species of Hydnum and Coprinus. Checking back through the immunization procedure there appeared to be no reasons for the difference that was apparent in the titer produced. However, when the extracts were being made, to be used as antigens, it was noticed that the smut protein was somewhat less soluble in the physiological salt than was the other proteins. This solubility property which existed may have carried through into the blood.

On Table V the results of the tests using the antiserum of Coprinus atramenterus are represented. All tests were begun, with the exception of that on U. zeae, with an antigen dilution of 1-100; it was not possible to start with a lower dilution because approximately only 1 per cent of the protein was extracted in the physiological salt. The only precipitation occurring, other than with the homologous antigen was that with Psalliota campestris which gave a positive reaction up to a dilution of 1-800. This was a rather unexpected result, because Psalliota, according to Engler & Prantl (4), and Gaumann and Dodge (5), appeared to be more closely related to Coprinus atramenterus than to Pleurotus, but still did not produce any precipitate. The morphological differentiation between the genus Coprinus and the

genus Pleurotus according to Engler-Prantl (4) is the spore color. However, according to the results obtained by the writer it would seem that spore color is not a characteristic which may be used to indicate phylogenetic relationship existing between these plants.

The antiserum of Coprinus comatus reacted strongly at a high dilution with its homologous antigen; but did not stimulate any precipitation whatsoever when it was brought in contact with heterologous antigen, as shown in Table VI. According to the results of Tables V and VI, it is possible to definitely distinguish by the precipitin test these two species of Coprinus.

It may be seen from the data in Tables VII and VIII, containing the results of the tests with antisera of the two species of Hydnum, that the different species tested could be separated from each other, due to the fact that the antigen did not precipitate with the antiserum of the other species. Since the organisms selected at random showed such precise differentiation, it is reasonable to assume that the phylogenetic relationship of other species may be just as clearly indicated.

In Tables IX, X, XI, XII, XIII, XIV, and XV all of the various antigens are tested with the antiserum of the different strains of U. zeae. No precipitation appeared outside of the homologous genera, but all of the strains of U. zeae gave similar reactions on the heterologous strains, making it apparent that it is not possible to determine the different

strains of this species from one another by serological means. This similarity in reactions was not due, as one might suppose, to foreign protein entering in by means of the media because tests were made to determine if the experimental animals had produced antibodies specific for the proteins that were contained in the media. According to the results obtained in these experiments it would favor the theory that the different strains are only phenotypic and that the forms change readily by a process of adaptation, rather than that they originate by hybridization and mutation.

Table V

Precipitin Reactions

Species	Dilution of the antigen extract							
	1-100	1-400	1-800	1-1600	1-2400	1-3200	1-4800	1-6400
Psalliota campestris	# ++ ++	+ ++	+- +	- -	- -	- -	- -	- -
Coprinus comatus	- -	- -	- -	- -	- -	- -	- -	- -
*Coprinus atromentarius	++++ ++++	+++ ++++	+++ ++++	++ +++	+ +++	+ ++	+- +	- -
Pleurotus ostreatus	- -	- -	- -	- -	- -	- -	- -	- -
Hydnum coralloides	- -	- -	- -	- -	- -	- -	- -	- -
Hydnum coput-ursi	- -	- -	- -	- -	- -	- -	- -	- -
Hydnum septentrionale	- -	- -	- -	- -	- -	- -	- -	- -
Ustilago zeae No. 1		- -	- -	- -				
Ustilago zeae No. 2		- -	- -	- -				
Ustilago zeae No. 3		- -	- -	- -				
Ustilago zeae No. 4		- -	- -	- -				
Ustilago zeae No. 5		- -	- -	- -				
Ustilago zeae No. 6		- -	- -	- -				
Ustilago zeae No. 7		- -	- -	- -				

*The antiserum of Coprinus atromentarius was used in this test.

#Signs in upper part of space are readings for 1st hour, lower part for 2nd hr.

Table VI

Precipitin Reactions

Species	Dilution of the antigen extract							
	1-100	1-400	1-800	1-1600	1-2400	1-3200	1-4800	1-6400
Psalliota campestris	# -	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
*Coprinus comatus	++++	++++	+++	++	+	+	+-	-
	++++	++++	++++	+++	++	++	+	-
Coprinus atromentarius	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Pleurotus ostreatus	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Hydnum coput-ursi	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Ustilago zeae No. 1		-	-	-				
		-	-	-				
Ustilago zeae No. 2		-	-	-				
		-	-	-				
Ustilago zeae No. 3		-	-	-				
		-	-	-				
Ustilago zeae No. 4		-	-	-				
		-	-	-				
Ustilago zeae No. 5		-	-	-				
		-	-	-				
Ustilago zeae No. 6		-	-	-				
		-	-	-				
Ustilago zeae No. 7		-	-	-				
		-	-	-				

*The antiserum of Coprinus comatus was used in this test

#Signs in upper part of space are readings for 1st hour, lower part for 2nd hour.

Table VII

Precipitin Reactions

Species	Dilution of the antigen extract							
	1-100	1-400	1-800	1-1600	1-2400	1-3200	1-4800	1-6400
Psalliota campestris	# -	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Coprinus comatus	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Pleurotus ostreatus	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Hydnum coput-ursi	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
*Hydnum septentrionale	++++	++++	+++	++	++	+	+-	-
	++++	++++	++++	+++	++	++	+	-
Ustilago zeae No. 1		-	-	-				
		-	-	-				
Ustilago zeae No. 2		-	-	-				
		-	-	-				
Ustilago zeae No. 3		-	-	-				
		-	-	-				
Ustilago zeae No. 4		-	-	-				
		-	-	-				
Ustilago zeae No. 5		-	-	-				
		-	-	-				
Ustilago zeae No. 6		-	-	-				
		-	-	-				
Ustilago zeae No. 7		-	-	-				
		-	-	-				

*The antiserum of Hydnum septentrionale was used in this test

#Signs in upper part of space are readings for 1st hour, lower part for 2nd hr.

Table VIII

Precipitin Reactions

Species	Dilution of the antigen extract							
	1-100	1-400	1-800	1-1600	1-2400	1-3200	1-4800	1-6400
Psalliota campestris	# - / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Coprinus comatus	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Coprinus atromentarius	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Pleurotus ostreatus	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
*Hydnum coralloides	++++ / ++++	++++ / ++++	+++ / ++++	++ / +++	+ / ++	+ / +	- / +	- / -
Hydnum coput-ursi	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Hydnum septentrionale	- / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -
Ustilago zeae No. 1		- / -	- / -	- / -	- / -	- / -		
Ustilago zeae No. 2		- / -	- / -	- / -	- / -	- / -		
Ustilago zeae No. 3		- / -	- / -	- / -		-		
Ustilago zeae No. 4		- / -	- / -	- / -				
Ustilago zeae No. 5		- / -	- / -	- / -				
Ustilago zeae No. 6		- / -	- / -	- / -				
Ustilago zeae No. 7		- / -	- / -	- / -				

*The antiserum of Hydnum coralloides was used in this test

#Signs in upper part of space are readings for 1st hour, lower part for 2nd hr.

Table IX

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# - / -	- / -	- / -	-	-	-
Coprinus atromentarius	- / -	- / -	- / -			
Hydnum coralloides	- / -	- / -	- / -			
Hydnum septentrionale	- / -	- / -	- / -			
*Ustilago zeae No. 1	+++ / ++++	++ / +++	+ / ++	+ / +	- / -	- / -
Ustilago zeae No. 2	+++ / ++++	++ / +++	+ / ++	+- / +	- / -	- / -
Ustilago zeae No. 3	+++ / ++++	++ / +++	+ / ++	+ / +	- / -	- / -
Ustilago zeae No. 4	+++ / ++++	++ / +++	++ / ++	+ / ++	- / +	- / -
Ustilago zeae No. 5	+++ / ++++	++ / +++	+ / ++	+- / +	- / -	- / -
Ustilago zeae No. 6	+++ / ++++	++ / +++	+ / ++	+ / +	- / -	- / -
Ustilago zeae No. 7	+++ / ++++	++ / +++	+ / ++	+- / +	- / -	- / -

*The antiserum of Ustilago zeae No. 1 was used in this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table X

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	-	-	-
	++++	+++	++	+	-	-
*Ustilago zeae No. 2	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 3	+++	++	+	+	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 4	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 5	+++	++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 6	+++	++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 7	+++	++	+	+	-	-
	++++	+++	++-	+	-	-

*The antiserum of Ustilago zeae No. 2 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table XI

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	+	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 2	+++	++	+-	+-	-	-
	++++	+++	++	+	-	-
*Ustilago zeae No. 3	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 4	+++	+++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 5	+++	++	+	+-	-	-
	++++	+++	++-	+	-	-
Ustilago zeae No. 6	+++	++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 7	+++	++	+	-	-	-
	++++	+++	++	+	-	-

*The antiserum of Ustilago zeae No. 3 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table XII

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 2	+++	++	++	+	-	-
	++++	+++	++	++	+-	-
Ustilago zeae No. 3	+++	++	+	+	-	-
	++++	+++	++	++	+	-
*Ustilago zeae No. 4	+++	++	++	+	-	-
	++++	+++	+++	++	+	-
Ustilago zeae No. 5	+++	++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 6	+++	++	++	+	-	-
	++++	+++	++	++	+-	-
Ustilago zeae No. 7	+++	++	+	+	-	-
	++++	+++	++	++-	-	-

*The antiserum of Ustilago zeae No. 4 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table XIII

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 2	+++	++	+	+	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 3	+++	++	+	+	-	-
	++++	+++	++-	+	-	-
Ustilago zeae No. 4	+++	++	+	+-	-	-
	++++	+++	++-	+	-	-
*Ustilago zeae No. 5	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 6	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 7	+++	++	+	+-	-	-
	++++	+++	++	+	-	-

*The antiserum of Ustilago zeae No. 5 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table XIV

Precipitin Reactions

Species	Dilutions of antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	+-	-	-
	++++	+++	++	++	-	-
Ustilago zeae No. 2	+++	++	++-	+	-	-
	++++	+++	+++	++	+	-
Ustilago zeae No. 3	+++	++	++-	+	-	-
	++++	+++	+++-	++-	+	-
Ustilago zeae No. 4	+++	++	++	+	-	
	++++	+++	++	++	+-	
Ustilago zeae No. 5	+++	++	+	+	+-	
	++++	+++	++-	++-	+	
*Ustilago zeae No. 6	+++	++	++	+	+-	
	++++	+++	+++	++	+	
Ustilago zeae No. 7	+++	++	++	+	-	
	++++	+++	+++	++	+-	

*The antiserum of Ustilago zeae No. 6 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

Table XV

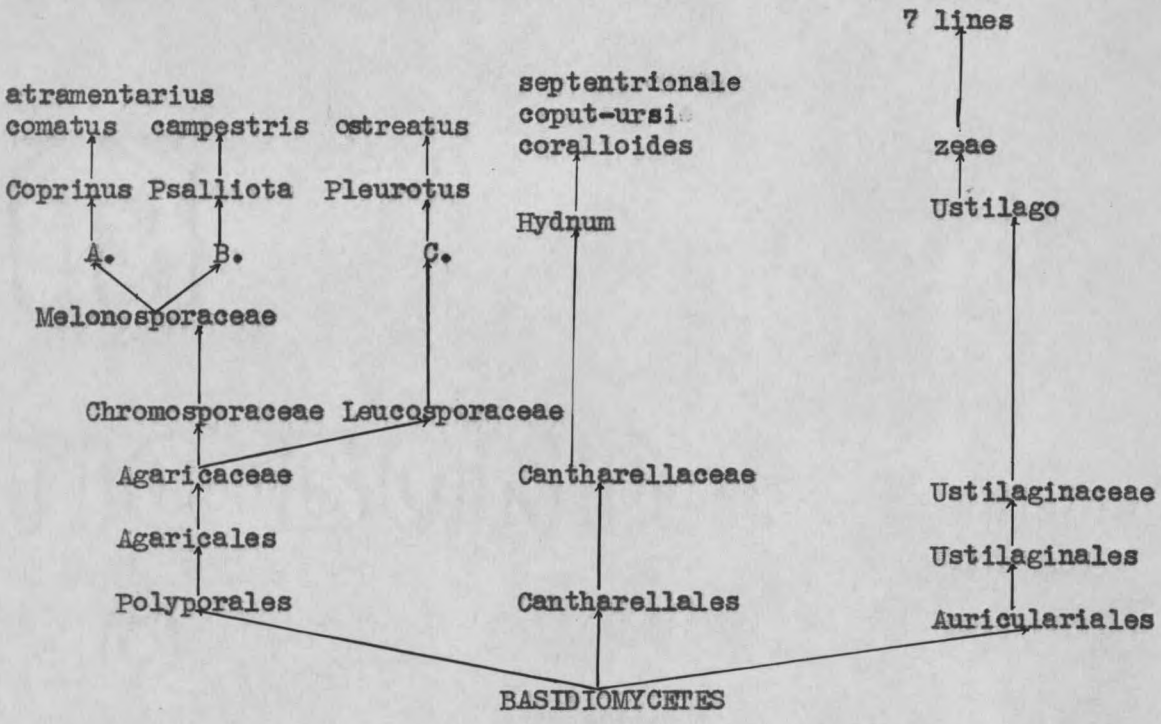
Precipitin Reactions

Species	Dilutions of Antigen					
	1-400	1-800	1-1600	1-2400	1-3200	1-4800
Coprinus comatus	# -	-	-	-	-	-
	-	-	-	-	-	-
Coprinus atromentarius	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum coralloides	-	-	-	-	-	-
	-	-	-	-	-	-
Hydnum septentrionale	-	-	-	-	-	-
	-	-	-	-	-	-
Ustilago zeae No. 1	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 2	+++	++	+	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 3	+++	++	+	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 4	+++	++	++-	+	-	-
	++++	+++	++	++-	-	-
Ustilago zeae No. 5	+++	++	++-	+-	-	-
	++++	+++	++	+	-	-
Ustilago zeae No. 6	+++	++	+	+	-	-
	++++	+++	++	+	-	-
*Ustilago zeae No. 7	+++	++	++	+-	-	-
	++++	+++	++	+	-	-

*The antiserum of, Ustilago zeae No. 7 was used for this test
#Signs in upper part of space are readings for 1st hr., lower part for 2nd hr.

GENERAL DISCUSSION

The following table, taken from Gaumann & Dodge (5), and a modification of that given by Engler & Prantl (4), will help to clarify the relationship of the Basidiomycetes used in this experiment.



The classification as indicated in the above table is based on morphological characteristics. The relationships given here are not in accordance with those presented by Mez (17) in his sero-diagnostic work on fungi. Although the work in this paper does not cover the identical field that is covered in Mez's work (17) the results tend to be in sympathy with his classification, because of the distant relationship that was apparent in the Hydnum, Cantharellales, Agaricaceae, and Ustilaginaceae.

Recently there has been extensive study on the physiologic specialization of U. zeae, because of its practical economic importance.

Christensen and Stakman (21) have carried out quite conclusively in their many experiments with U. zeae that in most cases new physiologic forms arise through mutation and hybridization. They based their assumption on the fact that they were able to derive distinct mutants from a single isolation made from a smut boil; all mutants retained their distinctive characters after repeated transfers. Frequently they were able to induce modifications in the lines of smut by altering the environmental conditions but all such variants revert back to the parental phenotype as soon as the causal stimulus was removed.

However, in the serological test carried out here, all the strains of U. zeae behaved as identical organisms, indicating that the specific proteins are the same in all strains of the smut tested. From this result it seems feasible to assume that the foregoing evidence would tend to support the older maintained theory that the environmental conditions are responsible for the occurrence of new physiologic forms of U. zeae, rather than the theory advanced by Stakman and Christensen (21).

Although there has not been a great deal of question concerning the relationship of the other Basidiomycetes studied in this paper, the results obtained gave some interesting information regarding the specific reaction occurring in the different species studied. These results also serve to substantiate the use of immuniological reactions as a correct and useful method by which to make phylogenetic classification of various biological groups.

SUMMARY AND CONCLUSIONS

1. Serological studies were made of seven strains of U. zeae, three species of Hydnum, two species of Coprinus, one species of Pleurotus, and one of Psalliota.

2. Due to the tendency of the smut spores to adhere to one another in a saline or oil solution it is not possible to administer the smut protein into the rabbits in this form.

3. The genera Psalliota, Pleurotus, Coprinus, Hydnum, and Ustilago were clearly distinguished from one another by serological means.

4. All of the species of Coprinus and Hydnum investigated by precipitin method were entirely specific in their antigenic characteristics, making it reasonable to assume that the relationship of other species may be just as clearly indicated.

5. In the serological test carried out on Ustilago zeae, all strains behaved as identical organisms.

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