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Studies in the Genetics and the Cytology of Ustilago avenae and Ustilago levis

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STUDIES IN THE GENETICS AND CYTOLOGY OF USTILAGO AVENAE AND USTILAGO LEVIS[1]

C. S. HOLTON[2]

INTRODUCTION

The two smuts of oats, loose smut caused by *Ustilago avenae* (Pers.) Jens. and covered smut caused by *U. levis* (K. and S.) Magn., occur wherever oats are grown. In some regions the loss caused by these smuts constitutes a major factor in the production of oats. Seed treatment is known to be an effective means of controlling both smuts of oats but, at its best, the method is undesirable in many respects, and it is generally conceded that the development of resistant varieties is the ideal solution to the oat smut problem. However, in breeding for resistance to oat smuts many complicating factors must be taken into consideration. In the first place, oat varieties may react differently (30) to the two species, thus making it necessary to deal with each separately. Consequently, the factors to be considered in planning and conducting a breeding program that aims at the development of smut-resistant varieties of oats are doubled because of the two species of the pathogen. Not only do two species of Ustilago cause oat smut but, in addition, there are many physiologic forms of each organism that must be taken into consideration in a breeding program, as varieties react differently to different forms. Furthermore, when a resistant variety is developed there is no assurance that it will always remain so, because the advent of a new physiologic form might at any time render a resistant variety completely susceptible. This has been demonstrated in the case of bunt-resistant crosses and varieties of wheat (17, 21, 22) and there seems to be no reason why it should not happen in smut-resistant varieties of oats. Therefore, if new or previously unknown forms of a pathogen affect resistant varieties of crop plants in this manner it is important to gain some information regarding the origin of new physiologic forms of the

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oat-smut fungi in order that a breeding program for smut-resistant varieties may be carried on to best advantage.

It has been clearly demonstrated (29, 30, 31, 32, 34) that *Ustilago avenae* and *U. levis* comprise different physiologic forms, or races, which attack the same varieties of oats with different degrees of virulence and differ also in their cultural characteristics. The exact origin of these forms has never been demonstrated, but it has been pointed out repeatedly that new forms of various pathogenic fungi might appear in a region as the result of (1) introduction from another region, (2) hybridization, and (3) mutation. These possibilities apply to the organisms causing oat smuts, as well as to others. The possibility of hybridization between physiologic forms within *U. avenae* and within *U. levis* is obvious and, in view of the striking similarities in the life cycles of the two species and their inevitable association in nature, it seems quite possible that hybridization might occur even between the two species. Considering these possibilities, there is undoubtedly ample opportunity for the production of new physiologic forms of either or both organisms causing oat smuts. Consequently, any plant-breeding program for oat-smut resistance should take these potentialities into consideration.

In addition to hybridization, mutation, altho possibly of minor importance, must be considered as a possible source of new physiologic forms of *Ustilago avenae* and *U. levis.* There have been numerous demonstrations of cultural variants, frequently designated as mutants, in pathogenic fungi, including certain smut fungi. Rodenhiser (32) describes a physiologic form of *U. avenae* that arose as a sector in a culture but, as he pointed out, the original culture was not of monosporidial origin and the sector might have been due to a separation of physiologic forms rather than to mutation. Other than this there seems to be no record of the production of new physiologic forms of the oat-smut fungi as a result of mutation.

It is obvious, therefore, that the plant breeder faces a difficult task in attempting to develop permanently smut-resistant oat varieties, particularly in view of the lack of information concerning the genetic behavior of the two smut-producing organisms and the large number of physiologic forms. It was with these considerations in mind that the studies reported herein were undertaken. Emphasis has been placed upon a study of the organisms as related to each other and to the origin of new physiologic forms.

The occurrence on the same host of two organisms that are so very similar in the fundamental details of their life cycles and yet so distinctly different in the external characteristics of their chlamydospores, offers a convenient opportunity for studying the nature of segregation of factors for cultural characteristics and the segregation and inheritance of

factors for chlamydospore characteristics. In *Ustilago avenae* the chlamydospores are echinulate; in *U. levis* they are smooth. In the original separation of these species the former was named as the causal organism of the loose smut of oats and the latter as the causal organism of the covered smut. However, in view of general observations made by the writer and conversations with various individuals, there seems to be some doubt as to the constancy of the gross morphological characters of diseased panicles, and it is questionable whether this characteristic should be used as a basis for separating the species. Altho it is possible to gain some indication as to the constancy of the association of *U. avenae* with the loose smut and *U. levis* with the covered smut by field observations and microscopic examination of miscellaneous collections of oat smut, it is obvious that by inoculating smut-free seed with crosses between monosporidial lines of known parentage more conclusive evidence should be gained as to the type of smut to be expected from each of the two species and crosses between them.

Sporidial fusions are frequently observed in *Ustilago avenae* and *U. levis* growing in culture, but no one has ever recorded observations of the various stages involved in this fusion process. Fusion of sporidia and the subsequent production of infection hyphae are necessary before smut is produced on the host. By observing the stages in the fusion process, the manner in which the act is accomplished, the length of time required for fusion and subsequent production of the infection hyphae, and the rate of growth of the infection hyphae, some valuable information in connection with the pathogenic properties of various combinations of monosporidial lines might be obtained. For example, various combinations of sexually opposite monosporidial lines might differ in the rate at which they fuse and the infection hyphae of different sporidial combinations might vary in their rate of growth. Considering such possibilities, it might be possible to formulate a basis for different pathogenic capabilities in various monosporidial crosses.

SPECIFIC OBJECTS OF THE INVESTIGATIONS

A. Genetics
   a. To determine the number of sexual groups in *Ustilago avenae* and *U. levis*.
   b. To study the process involved in the fusion of sporidia in culture with the view of determining the time required for completion of the process, the stages involved, and the subsequent development of fused sporidia.
   c. To determine whether *Ustilago avenae* and *U. levis* hybridize.
   d. To study the types of smutted panicles produced by crosses between monosporidial lines within and between the two species

with the view to determining the constancy of the association of *Ustilago avenae* with loose smut and *U. levis* with covered smut.

e. To determine whether new physiologic forms of *Ustilago avenae* and *U. levis* are produced by hybridization between species.

f. To study the nature of inheritance and segregation of chlamydospore characteristics in hybrid chlamydospores.

g. To study the nature of the segregation of cultural characteristics in *Ustilago avenae* and *U. levis.*

h. To determine the mutability of *Ustilago avenae* and *U. levis.*

B. Cytology

a. To determine the nuclear condition of *Ustilago avenae* and *U. levis* in certain stages of their development.

(1) Sporidia
(2) Promycelia
(3) Mycelium in culture
(4) Fusing sporidia

REVIEW OF LITERATURE

The genetics and cytology of the smut fungi, somewhat neglected by early investigators, have been studied extensively by recent ones. The progress of these investigations up to 1929 has been reviewed by Hanna (18) and Stakman *et al.* (37).

According to Hanna (18), numerous investigators had observed sporidial fusions in the smut fungi, but he states that Kniep was the first to demonstrate that these fusions take place only between certain sporidia. Kniep investigated *Ustilago violacea* (Pers.) Fuckel and demonstrated the existence of two kinds of sporidia, present in approximately equal numbers. Hanna (18) cites Dangeard, Harper, Lutman, Rawitscher, and Seyfert as contributors to our knowledge of the cytology of smut fungi in general. Thus, in 1929 Hanna (18) summarized the knowledge of the phenomena connected with the life cycle of smut fungi as follows: "In the smut fungi, therefore, a well-defined alternation of generations seems to occur. The mature chlamydospore contains a single nucleus. During the nuclear divisions which accompany the germination of the spore, a reduction takes place with the result that each promycelial cell and each sporidium receives a single haploid nucleus. In some species, the binucleate condition is initiated by the conjugation of sporidia or of promycelial cells, in others, e.g., *U. zeae,* by the conjugation of neighboring cells of the mycelium within the host plant. This binucleate condition persists until immediately before spore formation when the two nuclei in each cell fuse and chlamydospores once more are formed, each containing a single diploid nucleus." In his investigations Hanna (18) demonstrated the existence of four sexual

groups in *U. zeae* (Beckm.) Ung. and *Sorosporium reilianum* (Kühn) McAlpine. Christensen (9) obtained evidence of at least 24 different sexual groups in *U. zeae*.

Kniep (26) demonstrated that sporidia of different species of smuts fuse in culture, and Dickinson (12) has shown that *Ustilago levis* and *U. hordei* (Pers.) K. and S. in combination produced infection in oats, but he does not state that this infection resulted in the production of chlamydospores on the host.

Kämmerling (23) obtained infection with crosses between monosporidial lines of opposite sex of *Ustilago longissima* (Sow.) Tul. and *U. longissima* (Sow.) Tul. var. *macrospora* Davis and found that similar hybrids occur in nature. Furthermore, Hanna and Popp (20) have induced chlamydospore production by inoculating oat seedlings with crosses between monosporidial lines of *U. avenae* and *U. levis*. Flor (16) crossed *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn and the hybrid chlamydospores were smooth.

In studying the nature of segregation within *Ustilago hordei* and *U. levis,* Dickinson (13) found that cultural characters were segregated on a 2:2, 3:1, or 4:0 basis and that this segregation may take place in either of the two divisions of meiosis. He (12) found also that the segregation for sex factors in *U. levis* as well as in *U. hordei* was on a 2:2 basis, and Hanna and Popp (20) obtained similar results for *U. avenae* and *U. levis*. That segregation for sex factors is independent of segregation of factors for cultural characters has also been demonstrated by Dickinson (13) and he has shown (14) that the nuclear division in which segregation occurs may be affected by alteration of environmental conditions.

In an extensive discourse on mutation and hybridization in *Ustilago zeae,* Stakman et al. (37) state that Bauch was the first to demonstrate mutation in the smut fungi. In 1925 Bauch observed the phenomenon in *Ustilago bromivora* (Tul.) F. de W. and later this phenomenon has been observed in *U. zeae* by Christensen and Stakman (10) and Stakman et al. (37), and the latter pointed out that if the numerous variants observed by them in *U. zeae* were not the result of mutations they probably were due to some abnormal type of segregation. Recently Christensen (9) has presented further evidence of mutation in *U. zeae* and he states that "there seems to be no valid reason why the variants, which arise after many cultural generations, especially after the line has passed through one or two chlamydospore generations, should not be called mutants." He was unable to obtain culturally distinct lines of *U. zeae* by isolating successively produced sporidia from the same promycelial segment. However, he found that segregation for sex is sometimes extended to the next chlamydospore generation.

MATERIALS AND METHODS

The chlamydospore material used in these experiments was obtained from widely separated geographic regions. All cultures used were of monosporidial origin, and single sporidia were isolated according to the method described by Hanna (19). In making single sporidial isolations, each chlamydospore was given a number and the sporidia were numbered according to their respective positions on the promycelium, beginning at the apex. Thus, a culture labeled *U. avenae* 10-1 or *U. avenae* 10-2 originated from cell No. 1 and cell No. 2, respectively, of the promycelium of chlamydospore No. 10. When successively produced sporidia were isolated from the same segment, the resulting cultures were designated by letter in the order of isolation. For example, *U. avenae* 10-1a, -1b, -1c, etc. are cultures originating from cell No. 1 of the promycelium of chlamydospore No. 10 by isolating successively produced sporidia.

Potato-dextrose-agar, one per cent dextrose and 1.3 per cent agar, was used for studying the cultural characteristics of the various monosporidial lines. Plain agar was used for studying sporidial fusions and preparing inoculum. Nutrient-free medium seems to induce sporidial fusions at the expense of budding and it has the additional advantage of being unsuitable for the growth of contaminating organisms.

The sexual reaction of the various groups of monosporidial lines of *Ustilago avenae* and *U. levis* was determined by mixing the sporidia of the various groups of monosporidial lines in all possible combinations on plain agar. After several hours the mixtures were examined with the microscope to determine compatible and non-compatible crosses.

Two varieties of oats, Anthony and Liberty Hulless, known to be highly susceptible to smut, were used for inoculation experiments, and in almost all cases the Anthony seed was dehulled before germination and subsequent inoculation. The technic employed in making inoculations was, in general, as follows: Compatible crosses for inoculation (monosporidial lines of opposite sex) were made by mixing a mass of sporidia of each monosporidial line on plain agar in petri dishes, one cross to each dish, and spreading the mixture over the surface with a loop needle. At the end of 24 to 48 hours, fusion could be observed, and oat seedlings in which the coleoptile had completely emerged were placed in the inoculum and allowed to incubate for two days at 18-20° C., after which they were transplanted to the greenhouse, where the temperature remained at 18-20° C. throughout their development.

In view of the fact that all the inoculations were not made at the same time it is reasonable to expect that circumstances might necessitate varying the details of the technic from time to time, but the general principles remain the same in all cases. In presenting results, however, attention will be called to any pertinent variations from the technic outlined above.

EXPERIMENTAL RESULTS

GENETICS

Determination of sex groups

It was pointed out previously in this paper that Dickinson (12) found the segregation for sex factors to be on a 2:2 basis in *Ustilago levis* and *U. hordei,* while Hanna and Popp (20) found the same to be true in *U. avenae* and *U. levis.* In studying this phase of the problem the writer has isolated sets of four sporidia from six chlamydospores of *U. avenae* and nine of *U. levis.* These isolations were cultured on potato-dextrose-agar and the resulting monosporidial lines were paired in all possible combinations, within each group of four, on plain agar, for the purpose of determining their sexual behavior. The results of the matings between lines of *U. avenae* are summarized in Table 1, and those with *U. levis* in Table 2. An analysis of the data presented in Tables 1 and 2 indicates that the four sporidia on each promycelium belong to two sex groups. There are two possible arrangements of the two sex groups on the promycelium if reduction for sex occurs in the first meiotic division, and four possible arrangements if reduction occurs in the second division (see Table 3). There is no morphological distinction between the sex groups; therefore, in order to demonstrate their six possible arrangements on the promycelium it is necessary to mate all monosporidial lines from certain promycelia with those from other promycelia. As a result of crossing monosporidial lines within and between species, all six possible arrangements of the sex groups on the promycelium have been demonstrated in *U. levis* (Table 4). Altho only two arrangements of the sex groups on the promycelium were demonstrated in *U. avenae* (Table 1), it is probable that the entire six arrangements could be found if groups of four sporidia from a larger number of promycelia were to be properly mated. From the results of these experiments it is evident that there are two sex groups in *U. avenae* and *U. levis* and that they are arranged on the promycelia in a haphazard manner. This conclusion is in accordance with the results reported by other investigators (12, 13, 20).

Table 1

Results of Pairing Monosporidial Lines of Ustilago avenae Derived by Isolating Primary Sporidia from the Promycelia of Germinating Chlamydospores

U. avenae 10

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. avenae 17

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. avenae 24

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. avenae 22

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. avenae 30

	1	2	3	4
1	−	−	+	+
2	−	−	+	+
3	+	+	−	−
4	+	+	--	−

U. avenae 31

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

Table 2

Results of Pairing Monosporidial Lines of Ustilago levis Derived by Isolating Primary Sporidia from the Promycelia of Germinating Chlamydospores

U. levis 22

	1	3	2	4
1	−	−	+	+
3	−	−	+	+
2	+	+	−	−
4	+	+	−	−

U. levis 11

	1	2	3	4
1	−	−	+	+
2	−	−	+	+
3	+	+	−	−
4	+	+	−	−

U. levis 10

	1	3	2	4
1	−	−	+	+
3	−	−	+	+
2	+	+	−	−
4	+	+	−	−

U. levis 12

	1	2	3	4
1	−	−	+	+
2	−	−	+	+
3	+	+	−	−
4	+	+	−	−

U. levis 16

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. levis 39

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

Table 2—Continued

U. levis 13

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

U. levis 42

	1	3	2	4
1	−	−	+	+
3	−	−	+	+
2	+	+	−	−
4	+	+	−	−

U. levis 43

	1	4	2	3
1	−	−	+	+
4	−	−	+	+
2	+	+	−	−
3	+	+	−	−

Table 3

Possible Arrangements of the Two Sex Groups on the Promycelia of Ustilago avenae and U. levis, Segregating for Sex Factors

Sporidium No.	Reduction takes place in					
	1st division		2nd division			
1	A	B	A	B	A	B
2	A	B	B	A	B	A
3	B	A	B	A	A	B
4	B	A	A	B	B	A

Table 4

Six Arrangements of the Two Sex Groups in Ustilago levis as Found on Different Promycelia

Sporidium No.	Chlamydospore number					
	11	12	16	22	43	39
1	A	B	A	B	A	B
2	A	B	B	A	B	A
3	B	A	A	B	B	A
4	B	A	B	A	A	B

Process of Sporidial Fusion

A perusal of the literature pertaining to sporidial fusions in the smut fungi did not reveal any detailed descriptions of the actual steps involved in this process in *Ustilago avenae* and *U. levis*. Numerous statements have been made to the effect that sporidia of opposite sex in certain smuts must fuse before infection can take place. That sporidia of smut fungi fuse is stated in various mycological and plant pathological text-books but, curiously enough, the details of the process are invariably omitted. Bauch (6) describes two stages in the conjugation of sporidia in *Ustilago bromivora*. The first consists in the formation of a germ-tube by the sporidium and the second in its union with the germ-tube of

a sporidium of a different sex. He states further that germ-tubes are formed only in the presence of sporidia of the opposite sex, while the second phase occurs only under specific cultural conditions, such as result in the two germ-tubes being compressed together. Bauch (5) found also in *Ustilago longissima* and in its variety *macrospora* that the conjugating partners participate actively in the process of conjugation, each giving rise to a conjugation-tube. Dickinson (11) observed fusing hyphae in *U. hordei* and *U. levis* and he states that when two mycelia meet, each originating from a single sporidium, the hyphae either grow past each other indifferently or curve toward each other in a definite manner suggestive of chemotropic stimulation; on coming in contact they swell and the contents of one pass completely into the other, the resulting cell being termed a "fusion hypha." The description of sporidial and hyphal fusions in smut fungi, as outlined by these workers, does not apply to *U. avenae* and to *U. levis,* according to recent observations made by the writer; therefore a description of the process in these organisms is presented here.

Monosporidial lines of opposite sex were mixed and spread out thinly over poured plates of 2 per cent plain agar. Usually very young cultures were used in these studies and the development was observed with the high power of a microscope. Observations first were made to determine the length of time required for sporidia to fuse after lines of opposite sex had been mated. In one instance the sporidia began to fuse 35 minutes after they had been mixed, but in the majority of cases a period of one to two hours was necessary. Occasionally a period of three to four hours was necessary; hence it seems that the time limit between mating and fusing of monosporidial lines in *Ustilago avenae* and *U. levis* is quite variable. This fluctuation in the time limit is due, perhaps, to the particular lines used in the crosses, the age of the cultures, and environmental conditions. At any rate, it is evident that one must begin making frequent observations shortly after the sporidia have been mixed if all the steps involved in the process are to be observed.

The various steps observed in the fusing process between sporidia of *Ustilago avenae* and *U. levis* are shown in Plates 1 and 2, respectively. It is evident from the figures in Plates 1 and 2 that one sporidium of each fusing pair assumes an active rôle; the other remains passive, at least in the preliminary stages. The first development that can be observed is the appearance of a short projection near the tip of the active sporidium, which orients itself according to the relative location of the presumably nearest passive sporidium of opposite sex. The projection increases in length until it comes into contact with the passive sporidium near one end, and fusion follows. Immediately following fusion there is a definite and obvious increase in the length of the fusion tube, as evidenced by the distance between sporidia before and after fusion,

which undoubtedly accounts for the pronounced bending of fusion tubes and the relative positions of fused and non-fused ends of pairs of sporidia. For example, in Plate 2, Figure F, it is obvious that a decided increase in length of the fusion tube took place and caused the pronounced bend, since it was easier for the tube to bend than to force the sporidia farther apart. Also in Plate 1, Figures E and F, and M and N, the sporidia prior to fusing are lying parallel and after fusing the united ends are forced apart leaving the free ends as near to each other or nearer than they were originally. In some cases, as for example in Plate 2, Figure D, it is apparent that the fusing sporidia were not firmly attached to the substratum and were easily forced apart, thereby resulting in the production of a straight fusion tube. It is admitted that these explanations are highly speculative but they seem logical in view of the observations on the early stages of the fusion process. Furthermore, only sporidia in close proximity to each other appear to be able to fuse. Usually fusing sporidia in the early stages are separated by less than 5 μ, altho in one case observed two sporidia fused that were separated by a distance of 7 μ. A description of the fusion process, based on the recent observations reported above, might be briefly summarized as follows: One sporidium, designated as the active one, assumes a definite active attitude which is evidenced by the production of a short projection that grows in the direction of its nearest sexually opposite sporidium, which appears to be passive. When this projection comes into contact with the passive sporidium, fusion takes place and is immediately followed by the invariable increase in length of the fusion tube.

The fusion of two sporidia in *Ustilago avenae* and *U. levis* presumably initiates the dikaryophase, which is the parasitic stage of the organism and can not be propagated in culture. Immediately following completion of the fusion process, an infection hypha is produced which, in culture, is distinctly aerial in habit of growth and usually arises at some point along the fusion tube or, occasionally, from the non-fused end of one of the fused sporidia. This hypha grows quite rapidly for a period of time, varying from 18 to 36 hours with different hyphae, and finally reverts to the sporidial type of growth. Simultaneously with the production and subsequent growth of the infection hypha, the contents of the fused sporidia pass into the hypha and migrate toward the tip as the hypha increases in length. As the protoplasmic contents migrate with the growth of the hypha, cross walls appear in its evacuated basal portion. An indefinite number of these empty cells, usually from 3 to 5, immediately behind the protoplasm, remain rigid, but from that point back to the fusion tube the cells collapse, thereby apparently severing all cytoplasmic connection with the fused sporidia.

It has already been stated that the infection hyphae grow rapidly and some of them have been measured in order to determine the approxi-

mate rate of this growth. Eighteen pairs of fused sporidia were isolated before the infection hyphae had grown perceptibly . Eight of these pairs of fused sporidia were from a cross between *Ustilago avenae* 10-2 and *U. levis* 11-1 and the other ten were from a cross between *U. avenae* 10-3, and *U. levis* 11-2. Twelve hours after the isolations were made the infection hyphae were measured and the results are summarized in Table 5. As the table indicates, the rate of growth in the first cross ranged from 1.1 μ to 5.2 μ per hour, the average rate of growth for the eight hyphae in this group being 3.16 μ per hour. In the second group the growth of the hyphae ranged from 2.3 μ to 22.0 μ per hour, the average rate of growth for this group of ten hyphae being 9.03 μ per hour. It is noted that there is a wide difference in the rate of growth of the infection hyphae in the two crosses. This difference can not be accounted for at the present time, but possibly it was due to variation in environmental factors, such as the medium, the air, and the temperature. On the other hand, there is a possibility that this difference in rate of growth is manifested by various combinations of sexually opposite monosporidial lines, and this fact might account in part for different pathogenic capabilities in physiologic forms. However, this is merely a suggestion and not an attempt to draw conclusions from the limited data presented in Table 5.

Table 5

Rate of Growth of Infection Hyphae Produced from Pairs of Fused Sporidia in Crosses Between Ustilago avenae and U. levis*

Cross	Pair No.	Length of the fused spordia, u	Length of infection hyphae, u	Average growth per hour, u
U. avenae 10-2	1	7.0, 14.0	35	2.9
×	2	10.5, 15.0	35	2.9
U. levis 11-1	3	11.0, 11.5	63	5.2
	4	10.5, 11.5	14	1.1
	5	4.0, 14.0	42	3.5
	6	10.0, 12.0	38	3.1
	9	9.0, 14.0	38	3.1
	10	9.0, 14.0	42	3.5
			Average	3.16
U. avenae 10-3	11	10.5, 11.0	101	8.4
×	12	9.0, 11.0	70	5.8
U. levis 11-2	13	10.5, 14.0	264	22.0
	14	10.5, 12.5	66	5.5
	15	7.0, 14.0	233	19.4
	16	9.0, 10.5	50	4.1
	17	7.0, 14.0	171	14.2
	18	12.5, 14.0	28	2.3
	19	10.5, 10.5	49	4.1
	20	7.0, 7.5	54	4.5
			Average	9.03

* Measurements were made 12 hours after the fused pairs of sporidia had been isolated.

As stated previously, the infection hyphae are distinctly aerial in habit of growth and this characteristic, apparently constant in *Ustilago avenae* and *U. levis,* may be used as a definite criterion as to whether fusions have occurred in crosses between various monosporidial lines. Fusing sporidia and the resultant production of infection hyphae showing their aerial habit of growth are shown in Plate 3. The absence of sporidial fusions and infection hyphae in a cross between monosporidial lines of similar sex is shown in Plate 4.

According to Bauch (3, 4), sporidial lines of opposite sex in *Ustilago violacea* are readily determined by making various combinations between monosporidial lines and observing the type of growth in crosses between lines of opposite sex as compared with the type of growth in crosses between lines of similar sex or of monosporidial lines alone. Type of growth in crosses between lines of opposite sex differed very distinctly from that in crosses between lines of similar sex, and Bauch considered this a reliable method of making sex determinations in *U. violacea.* Tests have been made to determine whether this method could be used in the determination of sexually compatible and non-compatible monosporidial lines of *U. avenae* and *U. levis.* Sets of four monosporidial lines of *U. avenae* and *U. levis* originating from different promycelia have been paired in all possible combinations on poured plates of plain agar and also on potato-dextrose-agar. The results of some of these tests are photographically shown in Plates 5 and 6. In Plate 5 the cultures of *U. levis* are growing on potato-dextrose-agar and two of the monosporidial lines, 6 and 7, growing singly are very similar in type of growth to the combinations between lines in 3, 4, 8, and 9, in which fusions were observed to occur. On plain agar the growth was too scant for the detection of differences in type, but by examining the crosses microscopically it was possible to determine those in which sporidial fusions occurred. The type of growth on potato-dextrose-agar could then be compared on the basis of these determinations. Similar results have been obtained with monosporidial lines of *U. avenae,* but in one instance, as shown in Plate 6, four monosporidial lines of *U. avenae,* 1, 2, 9, and 10, were distinctly sporidial, while the compatible crosses, 4, 5, 6, and 8 were distinctly mycelial. Colonies 3 and 7, the non-compatible crosses, were intermediate between the sporidial and the mycelial type of growth. Thus, in this case, type of growth in fusing combinations seems to be sufficiently different from that of non-fusing ones for a determination of sexually opposite lines, but results were not obtained in all cases. Other tests of this nature in *U. levis* have yielded differences in type of growth between compatible and non-compatible crosses that paralleled the confirmatory observations made by microscopic examinations. However, the exceptions cited above are sufficient

to make this method unreliable for determining sexually compatible monosporidial lines in oat-smut fungi.

Hybridization

Altho Dickinson (12) obtained infection by inoculating oat seedlings with combinations of monosporidial lines of *Ustilago levis* and *U. hordei,* he does not state whether this infection produced smutty panicles. Hanna and Popp (20) produced smut on oat plants by inoculating seedlings with combinations of monosporidial lines of *U. avenae* and *U. levis.* The smut thus produced was of the loose type and the chlamydospores were echinulate. The writer has crossed, in all possible combinations, on plain agar, four monosporidial lines of *U. avenae* originating from chlamydospore 10 with four monosporidial lines of *U. levis* originating from chlamydospore 11. The results of these matings were determined by microscopic examinations and are summarized in Table 6. As indicated by the table, there were eight compatible combinations between the two sets of four monosporidial lines. It appears, therefore, (using sporidial fusions as a criterion) that the two species are perfectly interfertile.

Table 6

Sex Relationships Between Eight Monosporidial Lines of Ustilago avenae and U. levis Isolated from the Promycelia of Germinating Chlamydospores, as Indicated by Sporidial Fusions*

		U. avenae				*U. levis*			
		1	4	3	2	1	2	3	4
U. avenae	1	−	−	+	+	−	−	+	+
	4	−	−	+	+	−	−	+	+
	3	+	+	−	−	+	+	−	−
	2	+	+	−	−	+	+	−	−
U. levis	1	−	−	+	+	−	−	+	+
	2	−	−	+	+	−	−	+	+
	3	+	+	−	−	+	+	−	−
	4	+	+	−	−	+	+	−	−

* The numbers refer to the position of the sporidia on the promycelium, beginning at the apex.

Pathogenicity tests were made by inoculating Anthony oat seedlings with intra- and interspecific crosses. Inoculations were made also with monosporidial lines and with combinations of monosporidial lines of similar sex, as indicated by the absence of sporidial fusions in culture. Plants that were not inoculated served as checks.

The inoculation technic previously described was modified by adding 10 cc. of sterile distilled water to each culture, thus forming a suspension of the inoculum. Dehulled Anthony oat seedlings were placed in the inoculum and incubated at 18°-20° C. for 48 hours, after which they were transplanted to pots and incubated at the same temperature for

six days. The plants were then removed to the greenhouse and allowed to mature at a range of temperature from 18 to 20° C. The results of the inoculation are summarized in Table 7.

Table 7

Results of Inoculating Anthony Oats with Intra- and Interspecific Crosses Between Monosporidial Lines of Ustilago avenae and U. levis

Lines and crosses	Number of seedlings inoculated	Number of panicles smutted	Percentage of smut
U. avenae			
1	20	0	0.0
2	25	0	0.0
3	32	0	0.0
4	30	0	0.0
1 × 3	22	16	72.7
2 × 4	28	5	18.8
2* × 3	21	0	0.0
U. levis			
1	20	0	0.0
2	24	0	0.0
3	30	0	0.0
4	28	0	0.0
1 × 4	24	5	20.8
2 × 3	26	6	23.0
3* × 4	27	0	0.0
U. avenae × *U. levis*			
1 × 3	34	24	70.5
2 × 1	24	12	50.0
4 × 3	21	5	23.8
4 × 4	21	12	57.1
Check	75	0	0.0

* Combinations in which sporidia did not fuse in culture.

It is clear from Table 7 that *Ustilago avenae* and *U. levis* hybridize readily, as evidenced by the production of chlamydospores on the host. All combinations of monosporidial lines in which fusions were observed in culture produced chlamydospores on the host, the percentages of smutted panicles ranging from 23.8 to 70.5 in the interspecific crosses and from 18.8 to 72.7 in the intraspecific crosses. No smut resulted from inoculations with monosporidial lines along and with combinations of monosporidial lines that did not fuse in culture.

The chlamydospores produced by the interspecific crosses were echinulate and those produced by the intraspecific crosses had markings characteristic of the species. In this case, therefore, it appears that echinulation is dominant over smoothness. Furthermore, all smutted panicles produced by interspecific crosses were of the "loose" type, altho there was considerable variation in degree of looseness, which is in accordance with the results recently reported by Hanna and Popp (20). Usually the loose type of injury is attributed to *Ustilago avenae*, the species with echinulate spores. The intraspecific crosses produced the loose type of smut in *U. avenae* and the covered type in *U. levis*.

The experiment described above was repeated several times with the same general results. However, under greenhouse conditions oat plants develop rather poorly and the types of smutted panicles are not so typical as those produced in the field. Therefore, an additional experiment was made by inoculating oat seedlings with intra- and interspecific crosses of *Ustilago avenae* and *U. levis* and planting the seedlings in the field. In this case the seedlings were placed in a suspension of the inoculum, incubated 48 hours at 18-22° C., and then transplanted directly to the field, thus omitting the incubation period of six days used in the first set of inoculations. The types of smutted panicles produced by these crosses are indicated in Table 8. Four crosses within *U. avenae* were made and three of these produced the loose type of smut; the fourth cross (Plate 11) produced typical covered smut. The chlamydospores in all four crosses were echinulate. Fifteen crosses within *U. levis* were made and all of them produced the covered type of smut. The chlamydospores in all the crosses within *U. levis* were smooth. Twelve interspecific crosses were made and these produced both loose and covered smuts as well as intergrading types (Table 8). Four of the interspecific crosses produced covered smut, one cross produced loose smut, two crosses produced intermediate types, one cross produced both loose and covered types, one produced intermediate and covered types, and three crosses produced intermediate and loose types. From these results it seems apparent that there is no consistency with regard to the type of smutted panicles produced by crosses between *U. avenae* and *U. levis*. The loose and covered types, as well as intergrading types, may be produced by different crosses of the two species, as shown in Plate 7. The factor or factors determining the type of smutted panicle apparently have not been investigated. In one case two varieties, Anthony and Victory, were inoculated with the same cross and the smutted panicles of Anthony were typically covered; those of Victory were loose (Plate 8). This might indicate that varietal reaction determines, to some extent, the type of smutted panicle produced by interspecific crosses, altho in another experiment three varieties, Anthony, Liberty Hulless, and Victory, were inoculated with the same cross and the smutted panicles in all three varieties were of the covered type (Plate 9). Varietal reaction solely, therefore, does not appear to determine the type of smutted panicles produced. At any rate it is obvious that all interspecific crosses do not produce the same type of smutted panicles; nor does a given cross always produce only one type of smutted panicle, for in one interspecific cross both loose and covered types were produced in the same variety, Anthony (Plate 10). Furthermore, in one case *U. avenae* produced the covered type of smutty panicle, as shown in Plate 11; consequently the covered smut can not always be

attributed to *U. levis*. In no case, however, did a cross within *U. levis* produce any kind of smutted panicle other than the covered type.

Table 8

Types of Smutted Panicles Produced by Intra- and Interspecific Crosses of Ustilago avenae and U. levis on Anthony Oats Grown in the Field

Crosses		Type of smutted panicle	Crosses		Type of smutted panicle
U. avenae	1	Loose	*U. avenae* × *U. levis*		
	2	Loose		1	Covered
	3	Loose and covered		2	Covered
	4	Covered		3	Covered
U. levis				4	Covered
	1	Covered		5	Loose
	2	Covered		6	Intermediate
	3	Covered		7	Intermediate
	4	Covered		8	Loose and covered
	5	Covered		9	Intermediate and covered
	6	Covered		10	Intermediate and loose
	7	Covered		11	Intermediate and loose
	8	Covered		12	Intermediate and loose
	9	Covered			
	10	Covered			
	11	Covered			
	12	Covered			
	13	Covered			
	14	Covered			
	15	Covered			

The viability of chlamydospores resulting from interspecific crosses was determined by planting them on potato-dextrose-agar. The chlamydospores germinated in a perfectly normal manner, but primary sporidia that were isolated would not grow in culture, except in rare instances. More than 500 sporidia were isolated from chlamydospores of 20 interspecific crosses, but only seven monosporidial lines were obtained. Fortunately, however, both sex groups were represented in these seven lines and tests were made to determine their pathogenic capabilities.

The chlamydospores produced by crosses within *Ustilago avenae* and *U. levis* germinated normally and isolated sporidia developed in culture. Apparently, therefore, some lethal factor or factors may be present and become effective in crosses between these two species. Altho only 25 crosses have been made by the writer, the material used in the crosses was obtained from widely separated geographic regions and undoubtedly represents several physiologic forms. Consequently, it seems highly probable that the behavior of the relatively few interspecific crosses reported here is representative of what may occur in other crosses made under similar conditions.

The pathogenicity of the monosporidial lines originating from interspecific hybrids has been determined by crossing two monosporidial lines of opposite sex with each other and with other sexually opposite lines

of *U. avenae* and *U. levis*. The crosses with lines of *U. avenae* produced the loose type of smut, and the chlamydospores were echinulate, while the crosses with lines of *U. levis* produced the covered type of smut and in all cases the chlamydospores were smooth.

The cross between the two monosporidial lines originating from interspecific hybrid chlamydospores produced 25 per cent smut on Liberty Hulless oats and in this case the smut was buff instead of black, as is normal for oat smuts. The chlamydospores of the buff smut are smooth and, individually and collectively, they are hyaline. Germination tests proved that these chlamydospores germinate in the same manner as do *Ustilago avenae* and *U. levis*. The striking contrast between the buff and the normal types of oat smut is shown in Plate 12; the contrast between the color of individual chlamydospores of the two types of smut is clearly shown in Plate 13.

A complete set of primary sporidia was isolated from the promycelium of a germinating chlamydospore of the organism causing the buff smut and their sex reactions were determined by the usual method. Two sex groups were found to be present and inoculations were made to determine the pathogenicity of this organism. The results of the inoculations showed definitely that the life cycle of the organism producing the buff smut is identical with those of *Ustilago avenae* and *U. levis*. The smutted panicles intergrade between those with the outer glumes completely destroyed and those in which the glumes are not injured (Plate 14). The smutted kernels are always hard and brittle, however, and are not as easily crushed as those of the normal smuts. Soaking the smut balls of the buff smut in water causes them to become soft, and they are then easily crushed.

As far as the writer has been able to ascertain, this is the first recorded case of a type of oat smut that differs from *Ustilago avenae* and *U. levis,* altho Campagna (7) has reported the occurrence in nature of a white smut of wheat. The chlamydospores of this smut would not germinate; hence studies as to the nature and stability of this smut were not possible. The writer has never observed in nature the buff smut of oats described above, but its appearance is very inconspicuous and if it does occur in nature it might easily be overlooked. Even if it does occur in nature the extent of its occurrence is probably very limited, inasmuch as the smut balls are relatively hard and brittle, characteristics which would tend to restrict dissemination of the spores and establishment of the disease.

If this buff smut of oats is found in nature in the future, the question arises as to whether it should be given specific or varietal rank or neither. The organism and the disease produced by it resemble *Ustilago levis* and the covered smut in every respect except spore color, altho in some cases the outer glumes are destroyed. The smut balls,

however, are never of the loose type. The writer is of the opinion, therefore, that the organism causing the buff smut of oats should, if found in nature, be described as a variety of *U. levis.*

Segregation

For studying the inheritance of spore color in crosses between the "buff smut organism" and the common oat smut organisms, four monosporidial lines originating from the promycelium of a germinating chlamydospore of *Ustilago avenae* and four from *U. levis* were each crossed with four monosporidial lines originating from the promycelium of a germinating chlamydospore of the buff smut organism. Compatible crosses were determined on the basis of sporidial fusions in culture and inoculations were made accordingly. The inheritance of spore markings and spore color in intra- and interspecific crosses is summarized in Table 9 and illustrated diagrammatically in Plate 15a, b.

An analysis of the data presented in Table 9 and illustrated in Plate 15 reveals some rather interesting facts. Crosses between monosporidial lines within *Ustilago avenae* and *U. levis* produced echinulate and smooth spores, respectively, and in both species the spores were brown. When the two species were crossed the resulting chlamydospores were echinulate and brown, which is a definite indication that echinulation is dominant over smoothness. Virtually 100 per cent of these hybrid chlamydospores germinated normally, but the sporidia produced by them were almost 100 per cent non-culturable, i.e., they would not grow in culture. Of the relatively few monosporidial lines that originated from the hybrid chlamydospores, two, designated in Table 9 as "hybrid segregates," were crossed with each other, and this cross produced smooth, hyaline chlamydospores, designated in the table as the "buff smut organism." The inoculations were repeated and the same results were obtained. The production of smooth, hyaline chlamydospores by the hybrid segregates might be explained in one of two ways: Either the loss, probably through segregation, of the factor or factors responsible for echinulation and brown color or a difference between the factor or factors for spore color in *U. avenae* and in *U. levis.*

One of the hybrid segregates was crossed with four monosporidial lines of *Ustilago levis* and these crosses produced smooth, brown chlamydospores; the other hybrid segregate was crossed with two monosporidial lines of *U. avenae* and the resulting chlamydospores were brown and echinulate. Additional crosses of this kind could not be made because the cultures of the hybrid segregates were lost during a period of extreme summer heat in 1931. However, these results furnish additional proof that echinulation is dominant over smoothness and brownness is dominant over hyalineness.

Table 9

Results of Studies on the Inheritance of Chlamydospore Markings and Color in Crosses Between Monosporidial Lines Within and Between Ustilago avenae and U. levis and the "Buff Smut Organisms"

Crosses		Chlamydospore characteristics		Remarks
Kind	Number	Markings	Color	
U. avenae × *U. avenae*	10	Echinulate	Brown	Normal germination of chlamydospores and growth of sporidia in culture
U. levis × *U. levis*	16	Smooth	Brown	Normal germination of chlamydospores and growth of sporidia in culture
U. avenae × *U. levis*	25	Echinulate	Brown	Normal germination of chlamydospores. Less than 2 per cent of 500 isolated sporidia developed cultural lines
Hybrid segregates	1	Smooth	Hyaline	Germination of chlamydospores and growth of sporidia in culture typical of *U. avenae* and *U. levis*
Buff smut organism × Buff smut organism	8	Smooth	Hyaline	Germination of chlamydospores and growth of sporidia in culture typical of *U. avenae* and *U. levis*
Hybrid segregates × Buff smut organism	2	Smooth	Hyaline	Germination of chlamydospores and growth of sporidia in culture typical of *U. avenae* and *U. levis*
Hybrid segregates × *U. levis*	4	Smooth	Brown	Normal germination of chlamydospores. Sporidia 50 per cent viable in culture
Hybrid segregates × *U. avenae*	2	Echinulate	Brown	Normal germination of chlamydospores. Sporidia non-culturable
U. avenae × Buff smut organism	8	Echinulate	Brown	Normal germination of chlamydospores. Sporidia non-culturable
Buff smut organism × *U. levis*	8	Smooth	Brown	Normal germination of chlamydospores. Sporidia 50 per cent viable in culture
Buff smut organism × *U. levis* segregates	22	Smooth	Brown and hyaline	Brown dominant. 3:1 ratio.

Crosses between monosporidial lines within the buff smut organism produced smooth, hyaline chlamydospores through the third generation, thus proving the genotypic purity of this organism, at least insofar as spore markings and spore color are concerned. Furthermore, crosses between monosporidial lines of the buff smut organism and the hybrid segregates produced smooth, hyaline chlamydospores. This seems to be additional proof that these monosporidial lines (hybrid segregates) were pure for the two characteristics in question.

In eight crosses between four monosporidial lines of *Ustilago avenae* and four monosporidial lines of the buff smut organism all chlamydospores were echinulate and olive brown and germinated normally, but the sporidia would not grow in culture. These results are the same as those obtained with crosses between *U. avenae* and *U. levis*.

In eight crosses between four monosporidial lines of *Ustilago levis* and four of the buff organism all chlamydospores were smooth and brown. About 50 per cent of the primary sporidia isolated from these chlamydospores grew in culture. Usually two out of the four primary sporidia isolated from each promycelium grew in culture; altho, in a few cases none, or only one, of the four would grow. On the other hand, in one case all four sporidia from one promycelium grew. These results are indicative of a closer genetic similarity between the buff smut organism and *U. levis* than between the buff smut organism and *U. avenae*. Phenotypically the chlamydospores of the buff smut organism and those of *U. levis* are similar in that the chlamydospores of both are smooth, the only difference being in color.

Further evidence of a closer genetic similarity between the buff smut organism and *Ustilago levis* than between the buff smut organism and *U. avenae* is shown in Table 10. Crosses between the buff smut organism and *U. levis* produced, in general, higher percentages of smut than crosses between the buff smut organism and *U. avenae*. These results were confirmed by a second test using the same monosporidial cultures.

Table 10

Results of Inoculating Anthony Oats with Crosses Between Ustilago avenae and the Buff Smut Organism and U. levis and the Buff Smut Organism*

U. avenae × buff smut organism		*U. levis* × buff smut organism	
Cross	Percentage of smut	Cross	Percentage of smut
1 × 1	22.7	1 × 1	68.4
1 × 3	7.6	1 × 3	14.2
2 × 1	34.6	2 × 2	40.0
2 × 3	12.5	2 × 4	94.7
3 × 2	45.0	3 × 2	100.0
3 × 4	50.0	3 × 4	70.3
4 × 2	15.3	4 × 1	84.2
4 × 4	43.7	4 × 3	66.6

* The numbers indicate position of sporidia on the promycelium, beginning at the apex.

The segregation ratio of chlamydospore color was determined by crossing monosporidial lines derived from *U. levis* × buff smut organism chlamydospores. Twenty-two crosses were made and 17 produced smooth brown chlamydospores and 5 produced smooth hyaline chlamydospores, a 3 : 1 ratio. Four compatible combinations of monosporidial lines from the same promycelium produced smooth brown and hyaline chlamydospores in a perfect 3 : 1 ratio. These results clearly indicate the applicability of simple Mendelian principles in the genetics of oat-smut fungi.

It already has been stated that the factors of segregation for sex were found to be on a 2 : 2 basis and that all possible arrangements of the sex groups on the promycelium have been found, indicating that reduction for sex may occur either in the first or second meiotic division. Segregation of factors for cultural characteristics, such as color, topography, type of growth, and rate of growth, takes place in either division of meiosis in *Ustilago avenae* and *U. levis,* and segregation of these factors apparently is sometimes independent of segregation for sex factors. Dickinson (13) has found the same to be true for *U. levis.*

The nuclear division in which reduction takes place, according to Dickinson (14), may be influenced by altering the environmental conditions and this fact suggested the problem of determining whether segregation of factors for sex and cultural characteristics takes place beyond the second division of meiosis. Successively produced sporidia were isolated from the same segment of the promycelium in order to determine whether further segregation occurs in the individual segments. If further segregation does not occur in individual segments of the promycelium, successively produced sporidia should produce lines identical in their sexual and cultural reactions, unless mutation occurs.

Successively produced sporidia, ranging in numbers from 2 to 5, have been isolated from several promycelia, and in one case as many as 5 from each segment of the same promycelium. The monosporidial lines derived in this manner were compared culturally on potato-dextrose-agar in Ehrlenmeyer flasks. In almost every case in which two or more sporidia were isolated from the same segment these isolations produced two or more culturally different lines as shown in Plates 16, 17, and 18. All monosporidial lines originating from the same segment of a promycelium proved to be the same sex, however; hence it seems evident that in *Ustilago avenae* and *U. levis* segregation of factors for cultural characters may occur in the first or second divisions of meiosis, or later, while segregation of sex factors is complete in either the first or the second division.

Mutation

Cultural variants, originating as sectors in cultures of various fungi, including the smut fungi, have been designated as mutants (8, 10, 27, 32, 33, 35, 36, 37). In *Ustilago zeae* (Beckm.) Ung., for instance, Stakman *et al.* (37) have demonstrated that an indefinite number of culturally distinct lines may be obtained from certain monosporidial lines by isolating the sectors that occurred in each cultural generation. Many of these cultural variants showed pathogenic capabilities different from those of the parent and from each other. Thus, apparently there had been mutation for pathogenicity as well as for cultural characters.

In studying cultural characters of monosporidial lines of *Ustilago avenae* and *U. levis,* the writer has frequently observed cultural variants occurring in the form of sectors. A number of these sectors have been isolated and compared with the parent lines and with each other. In many cases these sector-produced lines have been culturally different from the parent line as well as from each other. Plate 19 shows distinct types of growth produced by lines of *U. avenae* that arose as sectors in a monosporidial line. Whether these cultural variants arose as a result of mutation or whether their origin is due to other causes is not definitely known. Possibly they are the result of delayed segregation. For example, as shown in Plate 20, four monosporidial lines of *U. avenae,* obtained by isolating the primary sporidia from the promycelium of a germinating chlamydospore, gave rise to several sectors that were isolated and compared with the original lines. Each of the four original lines was culturally distinct from the other three, and lines 1, 3, and 4 gave rise to sectors that produced lines that appeared culturally identical in almost every respect with line 2. Line 2 gave rise to a sector that produced a line similar to line 4. Line 1 gave rise to a sector that produced a line similar to line 3, and line 3 produced a sector-line similar to line 1. Lines 2 and 4 gave rise to sector-lines that reverted to the parental types. It can not be definitely stated that the cultural variants shown in Plate 20 did or did not result from mutation, but it would seem to be rather unusual for four monosporidial lines to give rise to one mutant each, all of which are virtually identical with each other and with one of the original lines, and for each of the four lines to give rise to mutants, each of which is similar to one or another of the original lines. In view of the fact that meiosis occurs in the promycelium of smut fungi, and since the four original lines pictured in Plate 20 originated from the same promycelium, it seems more probable that the variations described above and pictured in Plate 20 are the result of an extended or delayed reduction for cultural characters. Evidence has already been presented which indicates that segregation of factors for cultural characters within individual segments of the promycelium extends beyond the second division of meiosis. Furthermore, in view of

the evidence for an extended meiotic process in individual promycelial segments it seems very probable that segregation of factors for cultural characteristics might be extended through an indefinite number of nuclear divisions in monosporidial lines of *U. avenae* and *U. levis.* It seems possible, therefore, that cultural variants in *U. avenae* and *U. levis,* arising as sectors, may be due in many cases to some type of segregation rather than to mutation.

CYTOLOGY

A knowledge of the cytological phenomena in organisms used in genetical studies is of material aid in making the proper interpretation of results. Many investigators have contributed to our knowledge of the nuclear behavior in smut fungi. Nevertheless, in studying the genetics of an organism, it is always desirable to amplify these studies with cytological investigations. The writer has attempted, therefore, to determine the nuclear condition in *Ustilago avenae* and *U. levis* in various stages of their development.

Haidenhain's iron-alum haematoxylin is a satisfactory nuclear stain for this type of work and it was used in modification of the procedure followed by Hanna (18) in studying the cytology of *Ustilago zeae* and *Sorosporium reilianum.* The procedure may be briefly outlined as follows:

1. Spread a thin film of Mayer's fixative on a clean glass slide.
2. Place the material to be stained on the slide in a drop of distilled water and spread over the slide.[3]
3. Kill with fumes from Fleming's weaker solution; 10 to 20 minutes was the usual time employed.
4. Allow to dry 2 to 4 hours in the air.
5. Bleach in 10 per cent hydrogen peroxide to remove the black color caused by the osmic acid in Fleming's solution.
6. Wash off excess iron-alum in water bath.
7. Place the slide in 4 per cent iron-alum 8 to 12 hours.
8. Wash again in running water.
9. Stain in one-half per cent haematoxylin 24 hours.
10. Extract the stain with one per cent iron-alum. This process should be watched carefully with a microscope and allowed to continue until only the nuclei remain stained.
11. Wash in running water.
12. Dehydrate in a series of alcohols and pure xylol. The slides should be held in each solution about one minute.
13. Mount in balsam.

[3] Chlamydospores in the germinating stage were obtained by spreading them over the slide in one per cent malt extract and allowing them to reach the desired stage of germination before killing.

The sporidia of *Ustilago avenae* and *U. levis* are uninucleate, as shown in Plate 21. In fact, the nuclear condition in both species was the same in all the stages that were successfully stained. The mycelium that developed from monosporidial lines was uninucleate and sporidia that were budding from the promycelia of germinating chlamydospores were uninucleate, as well as the promycelial segments.

Attempts by the writer to stain fusing sporidia have been unsuccessful, but Allison (1) pictures a binucleate stage in fusing sporidia of *Ustilago hordei* and *U. levis*. He interprets this as being the initiation of the dikaryophase which persists throughout the parasitic stage. On the basis of the quotation from Hanna (18), the diploid phase is formed in the mature chlamydospores and when they germinate meiosis occurs, thus producing the haploid stage pictured in Plate 21.

DISCUSSION AND CONCLUSIONS

Apparently there are only two sex groups in *Ustilago avenae* and *U. levis*. The sporidia, carrying the sex factors, are arranged haphazardly on the promycelia of germinating chlamydospores. This is considered conclusive evidence that segregation of sex factors takes place in either the first or the second division of the diploid nucleus.

Hanna and Popp (20) suggested that *Ustilago avenae* and *U. levis* probably are closely related species, inasmuch as the sporidia fuse readily interspecifically and the resulting fusions produce smut on the host. Chlamydospores thus produced are echinulate, indicating that echinulation is dominant over smoothness. Similar results have been obtained by the writer. However, attempts to study the ratio of the segregation of factors for echinulate and smooth characters have been unsuccessful because sporidia produced by the hybrid chlamydospores will not grow in culture. Several hundred primary sporidia were isolated but, with rare exceptions, they failed to produce the cultural lines expected. These results are not surprising, however, in view of the well known fact that species hybrids in higher plants are, in many cases, highly sterile. The cause of sterility in interspecific hybrids apparently is not thoroly understood. Babcock and Clausen (2) point out that degree of sterility is not obviously correlated with other features, as, for example, enhanced vigor may be accompanied by complete fertility or complete sterility. Nevertheless, it seems well to point out the fact that *U. levis,* according to Dickinson (15), has 3 chromosomes in the haploid stage and *U. avenae,* as well as other species of Ustilago, according to Kharbush (24, 25), have 2 chromosomes in the haploid stage. Of course this difference in chromosome number can not be said to be responsible for the high degree of sterility in hybrids between *U. avenae* and *U. levis*. However,

it is a point that should not be disregarded in connection with a consideration of possible causes of the above mentioned hybrid-sterility.

The failure of hybrid sporidia to grow in culture while the sporidia of crosses between physiologic forms within the two species are readily cultured, serves to substantiate the original separation of the two organisms into distinct species. Also, the comparative behavior of the sporidia of species hybrids and those of crosses between physiologic forms seems to weaken the theory advanced by Rodenhiser (32) that "*U. avenae* and *U. levis* possibly are physiologic forms of the same species." He bases his conclusions on cultural characteristics, similarity in life cycles, certain chlamydospore characteristics, and the difficulty with which the two species sometimes are distinguished. These reasons seem to lose cogency in view of the results of genetic studies, which are perhaps more significant of relationship. Furthermore, Hanna and Popp (20) interpret their results as indicating that *U. avenae* and *U. levis* are genetically distinct with respect to the characters by which they are differentiated, but the ease with which crosses may be made between them suggests that they are closely related species. This conclusion apparently is based on the assumption that ability to cross is indicative of relationship between species, an assumption which is not entirely justifiable. In discussing species hybridization, Babcock and Clausen (2) state that "usually species standing close together in the taxonomic system cross readily and the difficulty increases as the species stand apart" but that "on the whole, crossability must be determined by trial, and the results frequently have little significance as indicative of relationship between species." They state further that "on the whole, sterility is the most characteristic feature of interspecific hybridization, and constitutes its most outstanding problem. If any one feature may be used as an index of relationship, the degree of sterility of the hybrid is perhaps most useful, but even it cannot be employed without due regard for details of individual cases." Therefore, it seems apparent that the suggestion made by Hanna and Popp (20) that *U. avenae* and *U. levis* are closely related species because they cross readily was based on a doubtful criterion of "close relationship" between species. The evidence presented in this paper does not necessarily prove a "distant relationship" between these two species; but if the degree of sterility in interspecific hybrids may perhaps be considered as the most useful index of relationship, then the high degree of sterility in hybrids between *U. avenae* and *U. levis* should be some indication of a relationship that can not be considered close. At any rate, regardless of whether it be "close" or "distant," the relationship between *U. avenae* and *U. levis* can not be determined on the basis of crossability. The writer is therefore inclined to consider the two oat smut organisms as distinct species

of undetermined relationship and not as physiologic forms of the same species.

The existence of physiologic forms in the oat-smut fungi and the demonstration of the origin of new physiologic forms of certain pathogenic fungi by hybridization and mutation (8, 10, 27, 28, 33, 35, 36, 37, 38) suggested the possibility that new forms of *Ustilago avenae* and *U. levis* might arise in like manner. However, the results of investigations on hybridization between the two species indicate that there is a rather limited possibility for the production of new physiologic forms by this means. Even tho the "buff smut organism" was produced as a result of segregation and recombination in the species hybrids, its production was accomplished under carefully controlled conditions such as might rarely appear in nature. Furthermore, the fact that this smut has never been found in nature seems to indicate that the proper conditions for its development rarely, if ever, occur in nature. Altho the possibility remains that new physiologic forms of *U. avenae* and *U. levis* may arise as a result of species hybridization, the probability seems very remote and the writer believes it can be disregarded in connection with a breeding program for the development of smut-resistant varieties of oats. More extensive investigations are necessary before conclusions can be drawn as to the probability of new forms arising through crossing between physiologic forms within species.

On the basis of the results reported here, there seems to be no consistency as to the type of smut produced by *Ustilago avenae* and interspecific crosses. Hanna and Popp (20) state that the interspecific crosses produced in the greenhouse were of the loose type, and the writer obtained similar results. However, when plants that were inoculated with various intra- and interspecific crosses matured in the field there was considerable variation in the type of smutted panicles, except in *U. levis,* which produced covered smut in every case. *U. avenae* produced loose, covered, and intergrading types of smutted panicles. One interspecific cross produced the loose and covered smuts on the same variety. One cross produced loose smut on one variety and covered smut on another variety; another cross produced the same type of smut on as many as three different varieties. This evidence tends to eliminate varietal reaction as a possible factor in determining the type of smutted panicle that will be produced. Just what the factors are that determine whether a potential smutted panicle will be of the loose or covered type have never been investigated but it is obvious, nevertheless, that gross morphological characters of the diseased host is an unreliable species distinction in the oat-smut fungi.

The production of a new type of smut with hyaline chlamydospores apparently afforded an opportunity for studying the inheritance and segregation of spore color in crosses between the brown types and the

hyaline type of chlamydospores. However, the same obstacle was encountered in these crosses that was encountered in the hybrids between *Ustilago avenae* and *U. levis*. The sporidia of the buff smut organism fused readily with those of *U. avenae* and *U. levis* and the chlamydospores produced on the host by these crosses were brown in color and echinulate and smooth, in crosses with *U. avenae* and *U. levis*, respectively. The chlamydospores produced by all these crosses germinated in a perfectly normal manner but it is important to note that none of the sporidia that were isolated from the crosses with *U. avenae* would grow in culture, while about 50 per cent of the sporidia isolated from chlamydospores of the crosses with *U. levis* grew in culture. It seems, therefore, that the genetic constitution of the buff smut organism is more similar to that of *U. levis* than to that of *U. avenae*. The only observable difference between the chlamydospores of the buff smut organism and those of *U. levis* is color, since in both organisms the spores are smooth. Just why 50 per cent of the sporidia are culturable and 50 per cent non-culturable in the crosses between *U. levis* and the buff smut organism is a matter of considerable speculation and no explanation will be offered at this time. It is readily concluded from the evidence presented in Table 9 and Plate 15 that the factors for echinulation and brown color are dominant over the factors for smoothness and hyalineness, and that brownness and hyalineness are segregated in a 3:1 ratio.

Altho the segregation of sex factors was found to be on a 2:2 basis, the factors for cultural characteristics do not segregate in so simple a manner. By isolating successively produced sporidia from the same segment of the promycelium it was found that two or more distinctly different cultural lines were obtained in almost every case. It seems evident, therefore, that segregation of factors for cultural characteristics is extended through several nuclear divisions. This is in decided contrast to the segregation of sex factors, which invariably was complete in either the first or the second nuclear division. The phenomenon of segregation in *Ustilago levis* has recently been considered in detail by Dickinson (15), and his results are clearly indicative of delayed segregation of factors for cultural characters.

The term "mutation" has been used frequently in referring to cultural variants occurring in various fungi. In view of the evidence presented in this paper it seems quite possible that cultural variants arising as sectors in *Ustilago avenae* and *U. levis* may be due, not to mutation, but to some type of segregation that results in the production of monosporidial lines of a heterogeneous rather than a homogeneous nature. The evidence presented in Plate 20 seems to be especially convincing that some type of abnormal segregation occurs in monosporidial lines of *U. avenae*.

The process of sporidial fusions in *Ustilago avenae* and *U. levis,* as observed in various stages, indicates a perceivable difference between male and female lines, since it is obvious that one sporidium is active while the other appears to be passive. This type of conjugation is suggestive of the conjugation process as observed in Mucor and it appears to be the first recorded case of this type of fusion in species of Ustilago, in so far as the writer has been able to ascertain from the available literature. Whether the active and the passive sporidia are segregated in a definite or a haphazard manner has not been determined.

According to the limited data presented on rate of growth of infection hyphae in two crosses between four monosporidial lines of *Ustilago avenae* and *U. levis,* there seems to be some indication of a decided difference in this phenomenon. Altho the evidence presented is not conclusive, it uncovers a possible field of research that might eventually result in an explanation of the contrast in virulence exhibited by different physiologic forms. If in certain physiologic forms less time is required for sporidia to fuse and the infection hyphae grow more rapidly than in another form, then it seems logical that the first form would be more capable of infecting and parasitizing the host than the second. Since little is known about the basis for resistance and susceptibility of different oat varieties to the various physiologic forms, the apparently meager evidence presented herein is considered sufficiently significant to warrant further investigation.

SUMMARY

1. Two sex groups were found in *Ustilago avenae* and *U. levis.* Segregation for sex is complete in either the first or the second meiotic division. These results substantiate the findings of others (12, 13, 20).

2. After mating monosporidial lines of opposite sex, the time required for fused sporidia to appear in culture varies from 35 minutes to several hours. The stages in the fusion process are: the production of a projection by one sporidium (the active sporidium) and the elongation of this projection until it comes into contact with a sexually opposite sporidium (the passive sporidium); fusion of the sporidia followed by a decided elongation of the fusion tube; and subsequent production of the "infection hypha."

3. *Ustilago avenae* and *U. levis* hybridize readily, as evidenced by sporidial fusions in culture and the production of chlamydospores on the host. Nevertheless, it seems better to consider them as distinct species and not physiologic forms of the same species.

4. Artificial inoculations with crosses between monosporidial lines of *Ustilago avenae* produced both the loose and covered smuts. *Ustilago levis* produced only the covered smut. Crosses between *U. avenae*

and *U. levis* produced loose, covered, and intergrading types of smut. Both the loose and covered types of smut may be produced on the same variety by the same cross and the same cross may produce loose smut on one variety and covered smut on another variety. On the other hand, the same cross may produce the same type of smut on as many as three different varieties.

5. The sporidia isolated from germinating hybrid chlamydospores were non-culturable, except in very rare instances. A cross between monosporidial lines from hybrid chlamydospores produced a buff smut, the chlamydospores of which are hyaline and smooth, which is a new and previously undescribed type of oat smut. The production of this new type of oat smut is some evidence that new physiologic forms of the oat-smut fungi may be produced by hybridization between the two species. However, in view of the high percentage of non-culturability of the hybrid sporidia the probability that this source of new physiologic forms of *Ustilago avenae* and *U. levis* will be a significant factor in the epiphytology of oat smuts seems very remote.

6. Hybrid chlamydospores are always echinulate, indicating dominance of echinulation over smoothness. Olive-brown color is dominant over hyalineness as indicated by the production of olive-brown chlamydospores by crosses between *Ustilago avenae* and the buff smut organism and between *U. levis* and the buff smut organism. Sporidia from chlamydospores produced by crosses between *U. avenae* and the buff smut organism were non-culturable but about 50 per cent of the sporidia isolated from chlamydospores produced by crosses between *U. levis* and the buff smut organism grew in culture. Inoculations were made with crosses between these sporidia and the results showed a segregating ratio of three brown to one hyaline.

7. Segregation of factors for cultural characteristics may extend indefinitely beyond the second nuclear division in meiosis. Successively produced sporidia from the same promycelial segment frequently develop distinctly different cultural lines. Evidence was obtained that segregation of factors for cultural characteristics takes place in monosporidial lines.

8. No concrete evidence for mutation in *Ustilago avenae* and *U. levis* was obtained. Cultural variants appearing as sectors in monosporidial lines are interpreted as being the result of segregation.

9. The sporidia of *Ustilago avenae* and *U. levis* are uninucleate. Each promycelial cell and primary sporidium is uninucleate. The mycelium of monosporidial cultures is uninucleate. Attempts to stain fusing sporidia were unsuccessful, but according to Allison (1) fused sporidia initiate the dikaryophase, which is binucleate. This stage persists throughout the parasitic stage to the production of chlamydospores which, when mature, have a single diploid nucleus.

LITERATURE CITED

1. Allison, C. C. Sex Relationships in Some of the Cereal Smuts. Unpublished thesis. University of Minnesota, 1930.
2. Babcock, E. B. and R. E. Clausen. Genetics in Relation to Agriculture. 675 pp. McGraw-Hill Book Company. New York. 1927.
3. Bauch, R. Kopulationsbedingungen und Sekundäre Geschlechtsmerkmale bei *Ustilago violacea*. Biol. Centralbl. **42**: 9-38. 1922.
4. ————————. Rassenunterschiede und Sekundäre Geschlechtsmerkmale beim Antherenbrand. Biol. Centralbl. **47**: 370-383. 1927.
5. ————————. Ueber *Ustilago longissima* und ihre Varietät *macrospora*. Ztschr. Bot. **15**: 241-279. 1923.
6. ————————. Untersuchungen über die Entwickelungsgeschichte und Sexualphysiologie der *Ustilago bromivora* und *Ustilago grandis*. Ztschr. Bot. **17**: 129-177. 1925.
7. Campagna, E. New White Smut of Wheat Collected at Sainte-Anne-de-la-Pocatiére. Ann. Rept. Que. Soc. Prot. Plants **18**: 71-72. 1926.
8. Christensen, J. J. Physiologic Specialization and Mutation in *Helminthosporium sativum*. Phytopath. **15**: 785-795. 1925.
9. ————————. Studies on the Genetics of *Ustilago zeae*. Phytopath. Ztschr. **4**: 129-188. 1931.
10. ———————— and E. C. Stakman, Physiologic Specialization and Mutation in *Ustilago zeae*. Phytopath. **16**: 979-999. 1926.
11. Dickinson. S. Experiments on the Physiology and Genetics of the Smut Fungi. Hyphal fusion. Proc. Roy. Soc. London, Ser. B. **101**: 126-136. 1927.
12. ————————. Experiments on the Physiology and Genetics of the Smut Fungi. Seedling infection. Proc. Roy. Soc. London. Ser. B. **102**: 174-176. 1928.
13. ————————. Experiments on the Physiology and Genetics of the Smut Fungi. Cultural characters. 1. Their permanence and segregation. Proc. Roy. Soc. London, Ser. B. **103**: 547-555. 1928.
14. ————————. Experiments on the Physiology and Genetics of the Smut Fungi. Fifth Internat. Bot. Congress. Absts. of Communications, p. 237. 1930.
15. ————————. Experiments on the Physiology and Genetics of the Smut Fungi. Cultural characters. 2. The effect of certain external conditions on their segregation. Proc. Roy. Soc. London. Ser. B. **108**: 395-423. 1931.
16. Flor, H. H. Heterothallism and Hybridization in *Tilletia tritici* and *Tilletia levis*. Jour. Agr. Res. **44**: 49-58. 1932.
17. Gaines, E. F. New Physiologic Forms of *Tilletia tritici* in Wheat. Phytopath. **18**: 579-588. 1928.
18. Hanna, W. F. Studies in the Cytology and Physiology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. **19**: 415-442. 1929.
19. ————————. A Simple Apparatus for Isolating Single Spores. Phytopath. **18**: 1017-1021. 1928.
20. ————————, and W. Popp. Relationship of the Oat Smuts. Nature **126**: 843-844. 1930.
21. Holton, C. S. A Probable Explanation of Recent Epidemics of Bunt in Durum Wheats. Phytopath. **20**: 353-357. 1930.
22. ————————. The Relation of Physiologic Specialization in Tilletia to Recent Epiphytotics of Bunt in Durum and Marquis Wheats. Phytopath. **21**: 687-694. 1931.

23. Kämmerling, Hans. Über Geschlechterverteilung und Bastardierung von *Ustilago longissima* und ihrer Varietät *macrospora*. Ztschr. Bot. **22**: 113-142. 1929.
24. Kharbush, S. S. Contribution à l'étude des phénomènes sexuels chez les Ustilaginées. Ann. Sci. Nat. Bot., Ser. 10. **9**:285-297. 1927. (Abst.) Rev. Appl. Myc. **7**: 532. 1928.
25. ————. Recherches histologiques sur les Ustilaginées. Rev. Path. Vég. et Ent. Agr. **15**: 48-56. 1928. (Abst.) Rev. Appl. Myc. **7**: 532-533. 1928.
26. Kniep, H. Über Artkreuzungen bei Brandpilzen. Ztschr. Pilsk. N. S. **5**: 217-247. 1926.
27. Newton, Margaret and Thorvaldur Johnson. Color Mutations in *Puccinia graminis tritici*. (Pers.) Erikss. and Henn. Phytopath. **17**: 711-725. 1927.
28. ————, ————, and A. M. Brown. A Preliminary Study of the Hybridization of Physiologic Forms of *Puccinia graminis tritici*. Scien. Agr. **10**: 721-731. 1930.
29. Reed, George M. Physiologic Races of Oat Smuts. Amer. Jour. Bot. **11**: 483-492. 1924.
30. ————. Further Evidence of Physiologic Races of Oat Smuts. Mycologia **19**: 21-28. 1927.
31. ————. New Physiologic Races of the Oat Smuts. Bull. Torrey Bot. Club **56**: 449-470. 1930.
32. Rodenhiser, H. A. Physiologic Specialization in Some Cereal Smuts. Phytopath. **18**: 955-1003. 1928.
33. ————. Physiologic Specialization and Mutation in *Phlyctaena linicola*. Phytopath. **20**: 931-942. 1930.
34. Sampson, Kathleen. Some Infection Experiments with Loose and Covered Smuts of Oats which Indicate the Existence in Them of Biological Species. Ann. Appl. Biol. **12**: 314-325. 1925.
35. Stakman, E. C., J. J. Christensen, and W. F. Hanna. Mutation in *Ustilago zeae*. (Abst.) Phytopath. **19**: 106. 1929.
36. ————, M. N. Levine, and R. U. Cotter. Hybridization and Mutation in *Puccinia graminis*. (Abst.) Phytopath. **20**: 113. 1930.
37. ————, J. J. Christensen, C. J. Eide, and B. Peturson. Mutation and Hybridization in *Ustilago zeae*. I. Mutation; II. Hybridization. Minn. Agr. Expt. Sta. Tech. Bull. 65. 1929.
38. ————, M. N. Levine, and R. U. Cotter. Origin of Physiologic Forms of *Puccinia graminis* Through Hybridization and Mutation. Scien. Agr. **10**: 707-720. 1930.

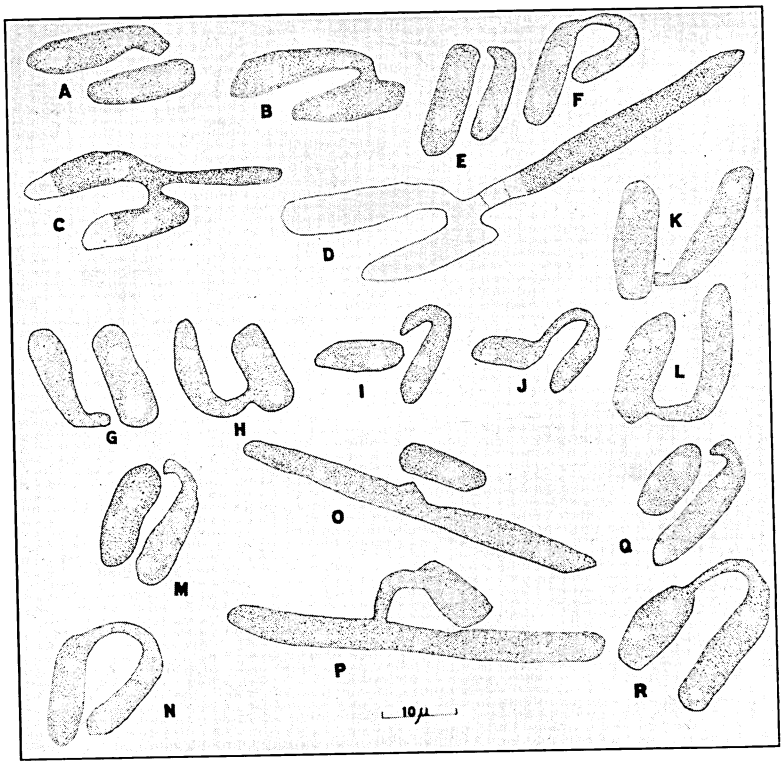


Plate 1. Camera-Lucida Drawings of Fusing Sporidia of *Ustilago avenae* Showing Stages Involved in the Fusion Process. × 2200

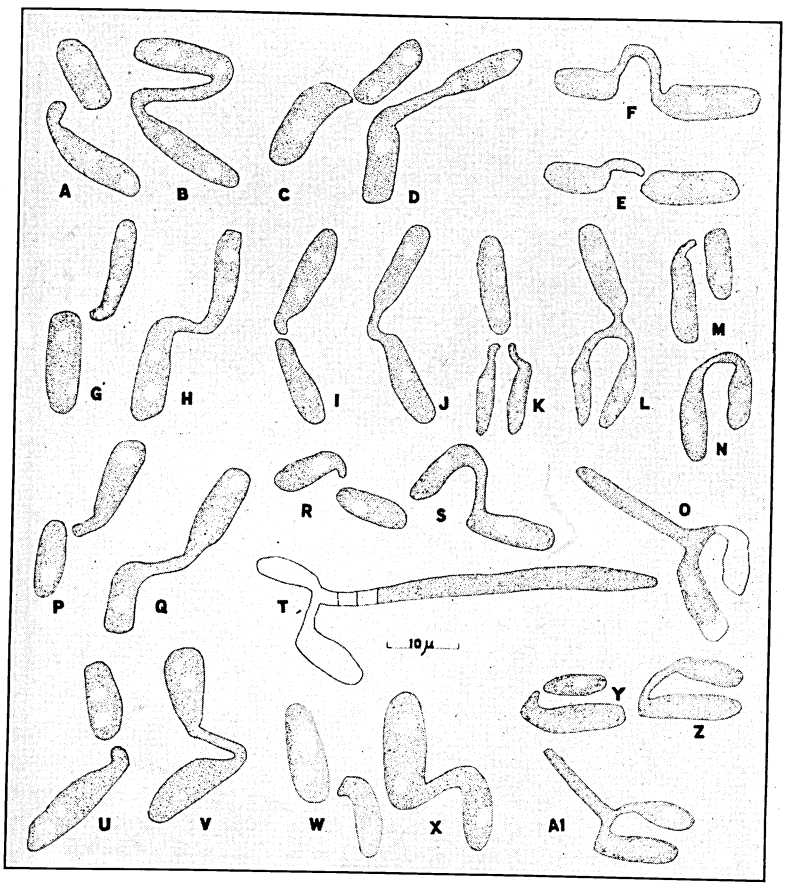


Plate 2. Camera Lucida Drawings of Fusing Sporidia of *Ustilago levis* Showing Stages Involved in the Fusion Process. × 2200

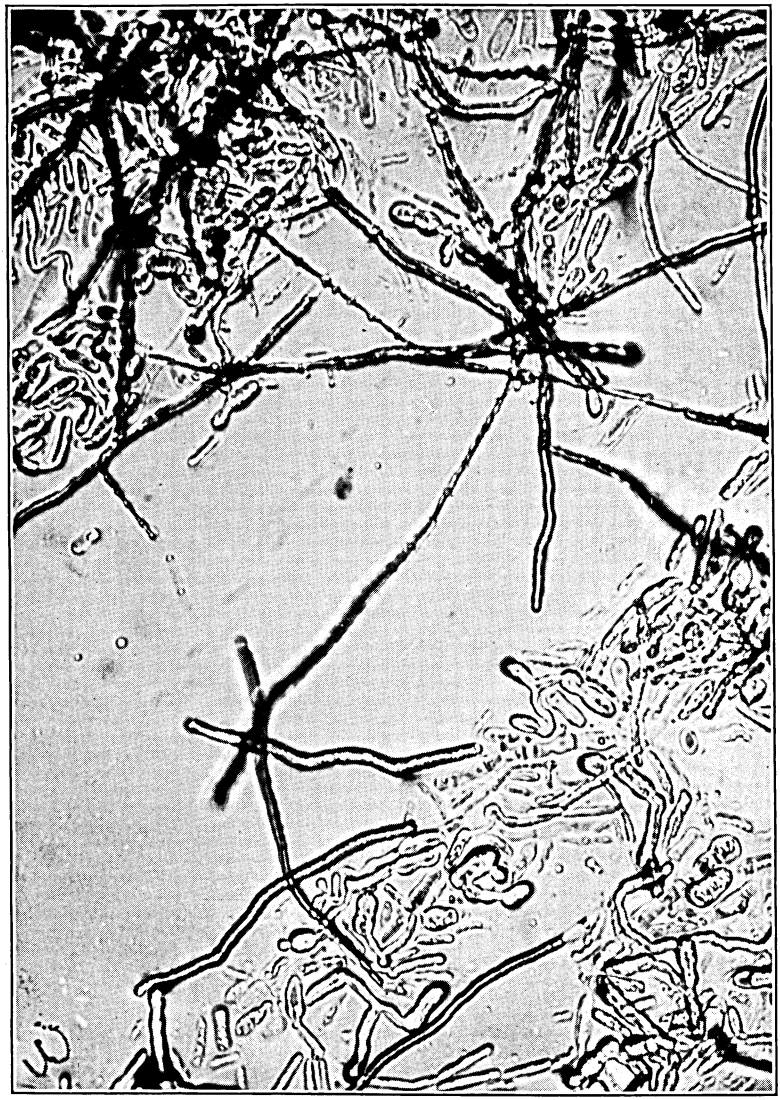


Plate 3. Photomicrograph Showing Fusing Sporidia and Aerial Hyphae of *Ustilago levis*, Observed Only in Cultures Containing Sporidia of Both Sex Groups

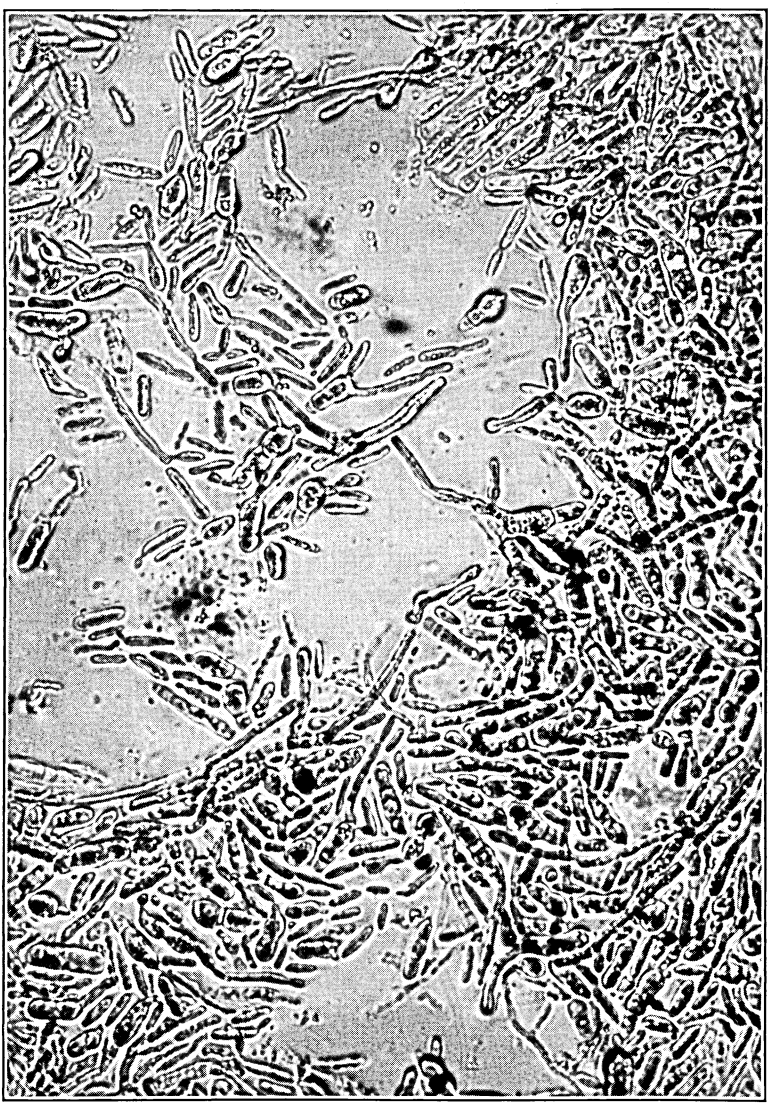


Plate 4. Photomicrograph of a Mixture of Two Monosporidial Lines of *Ustilago levis* of Similar Sex Showing Absence of Sporidial Fusions and Aerial Hyphae

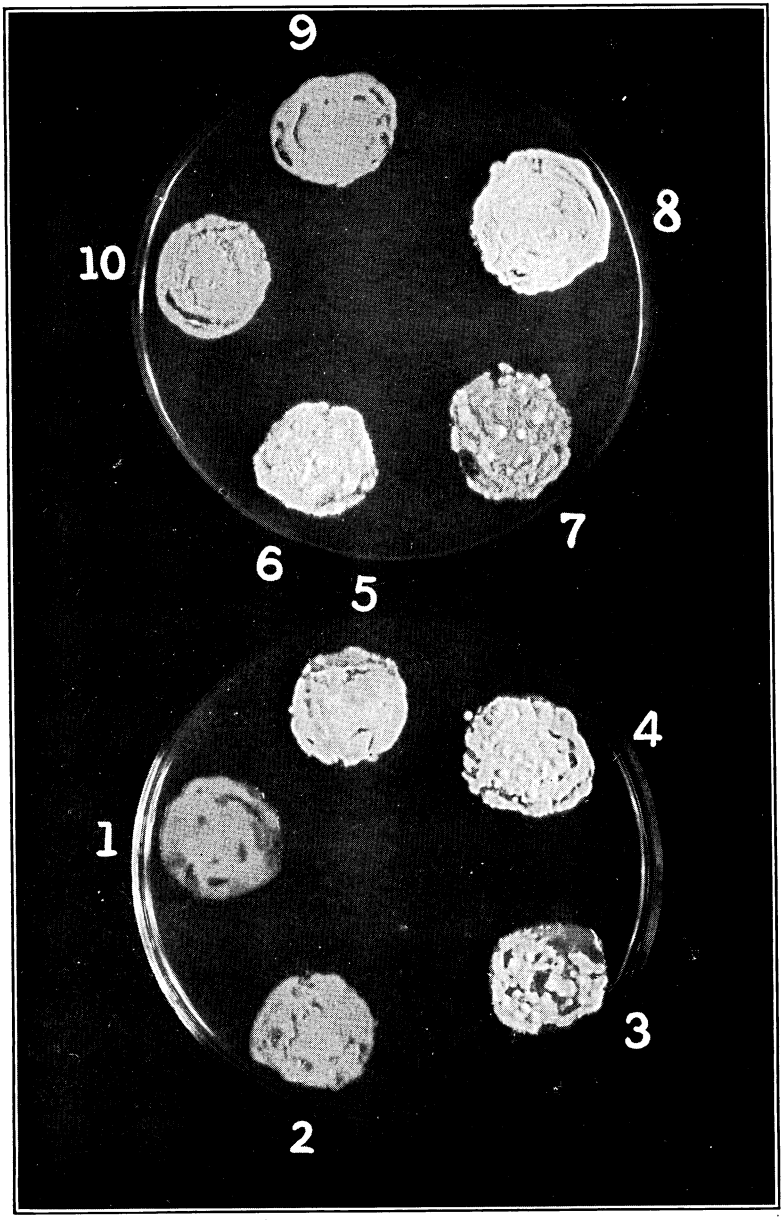


Plate 5. Type of Growth Produced by Four Monosporidial Lines of *Ustilago levis* and All Possible Crosses Between Them

1, 2, 6, and 7 are the monosporidial lines, 3, 4, 8, and 9 are compatible crosses, and 5 and 10 are non-compatible crosses.

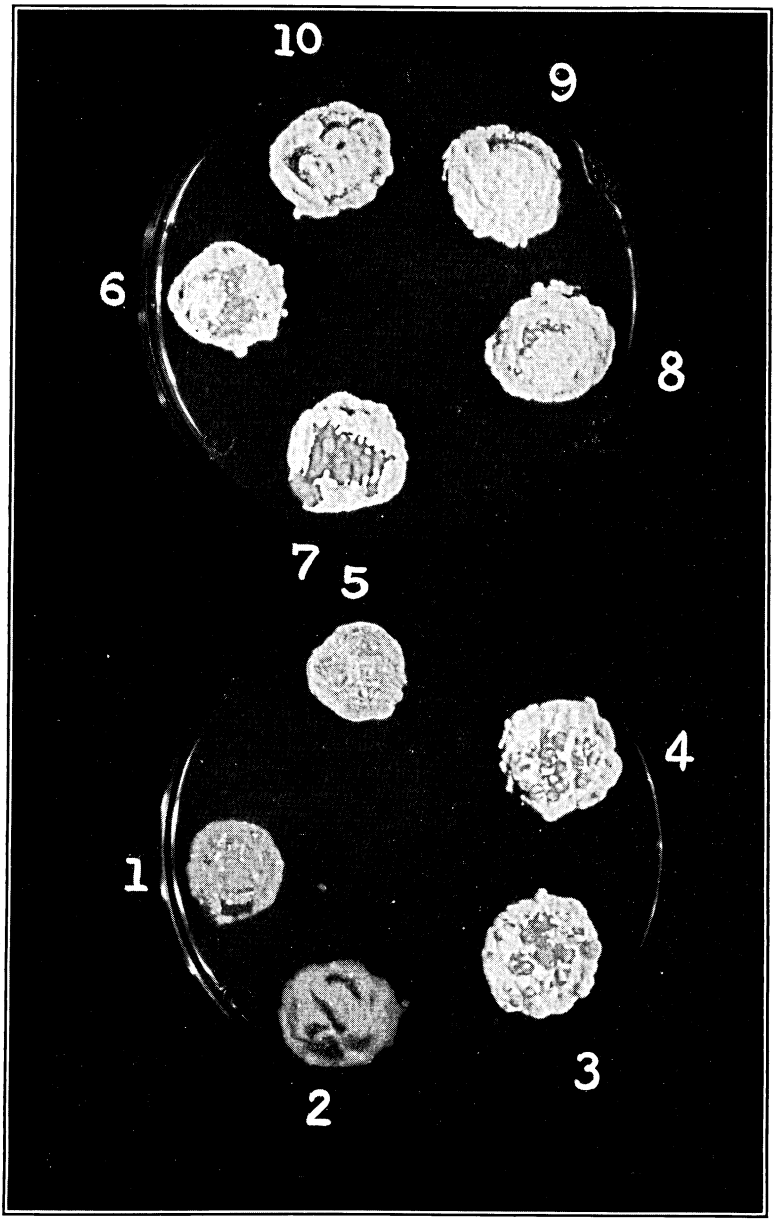


Plate 6. Type of Growth Produced by Four Monosporidial Lines of *Ustilago avenae* and all Possible Crosses Between Them

1, 2, 9, and 10 are the monosporidial lines; 4, 5, 6, and 8 are compatible crosses, and 3 and 7 are non-compatible crosses.

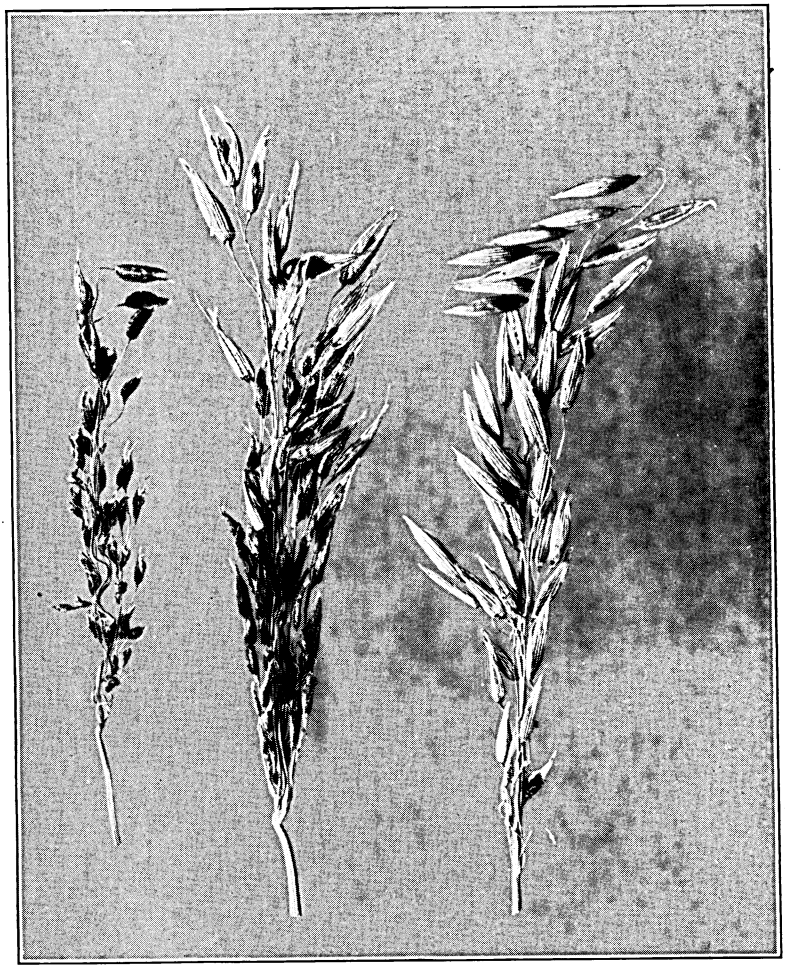


Plate 7. Loose, Intergrading, and Covered Types of Smut on Anthony Oats Produced by Three Different Crosses Between *Ustilago avenae* and *U. levis*

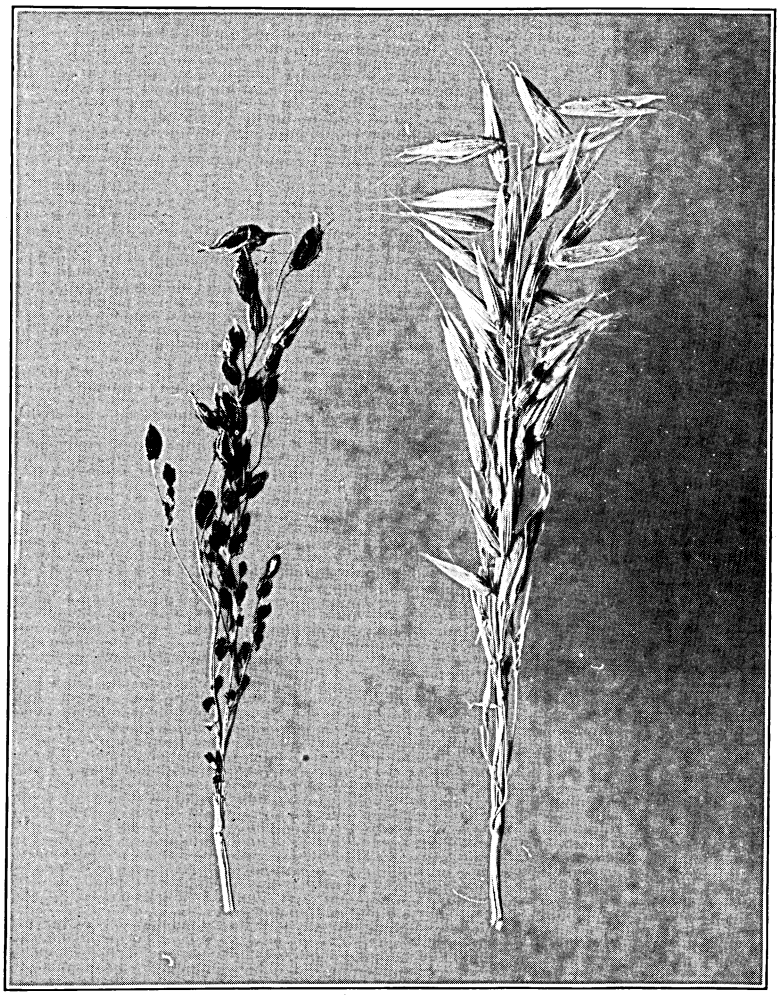
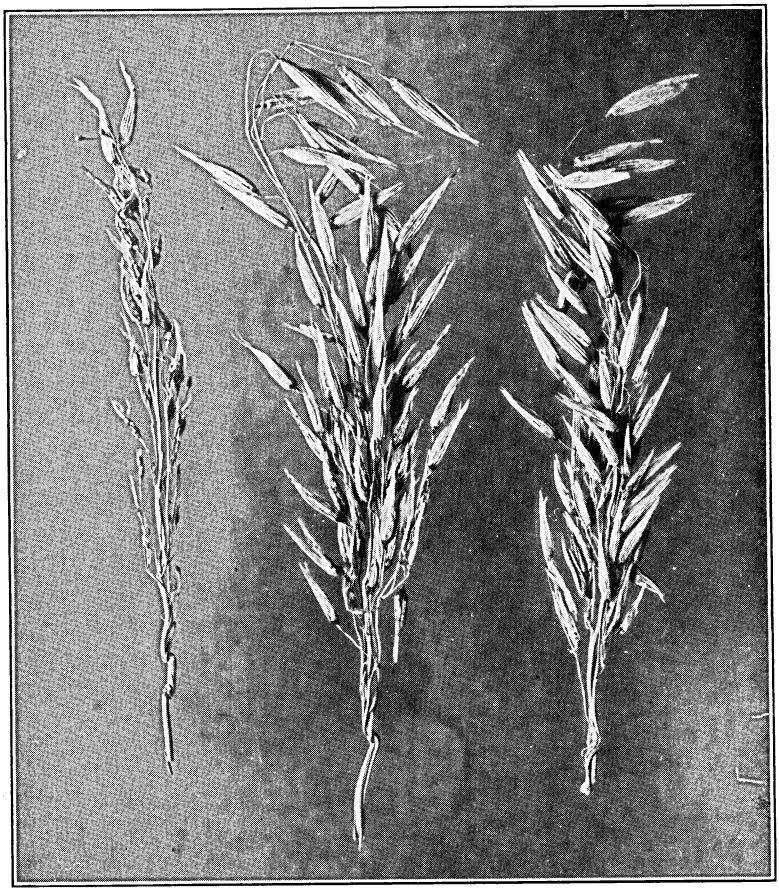


Plate 8. Loose Smut on Victory and Covered Smut on Anthony Oats Produced by a Cross Between the Buff Smut Organism and *Ustilago levis*



A B C

Plate 9. Covered Smut on (A) Victory, (B) Anthony. and (C) Liberty Hulless Oats Produced by a Cross Between *Ustilago avenae* and *U. levis*

Plate 10. Loose and Covered Smut on Anthony Oats Produced by a Cross Between *Ustilago avenae* and *U. levis*

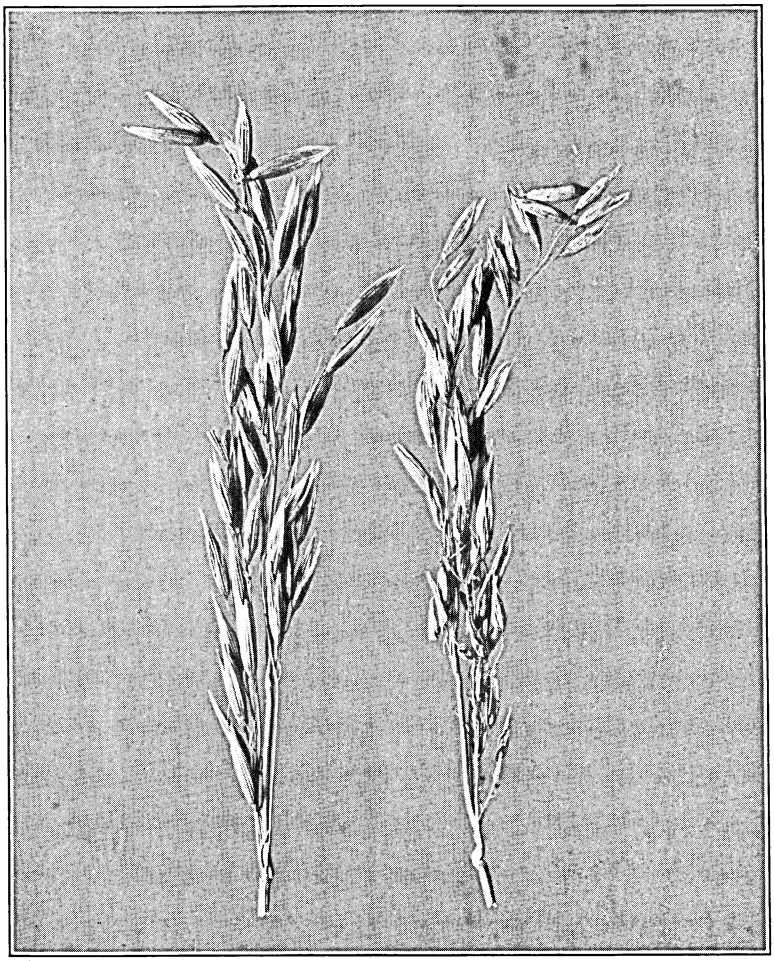
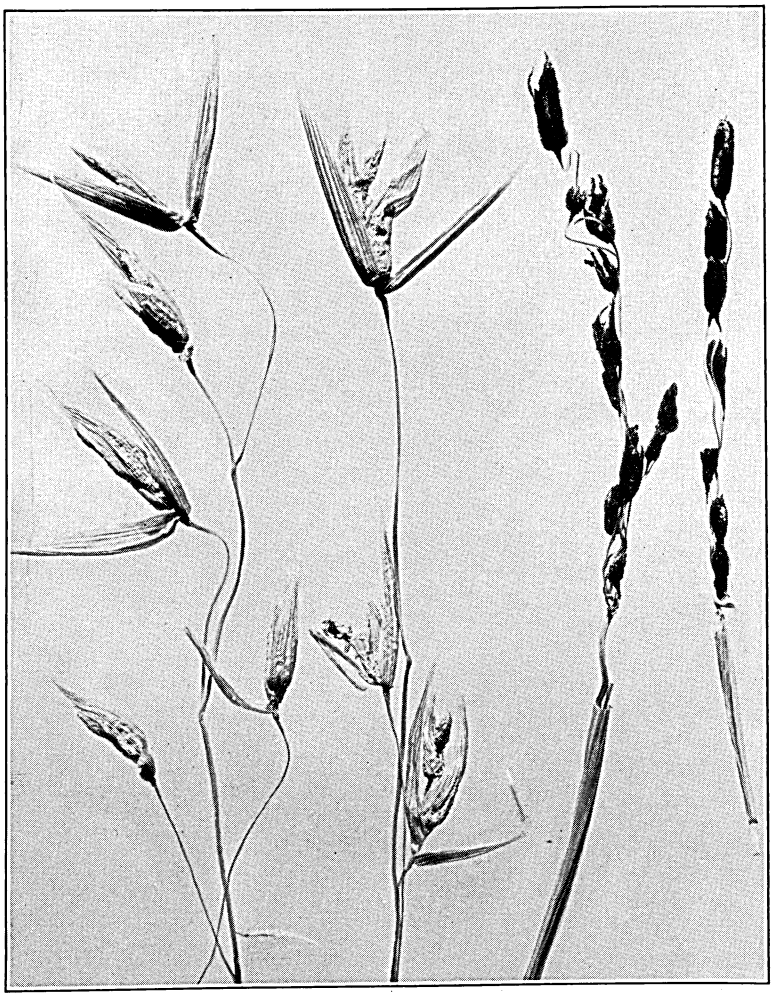


Plate 11. Covered Smut on Anthony Oats Produced by *Ustilago avenae*



A B

Plate 12. Color Contrast Between the Buff (A) and the Common (B) Oat Smut

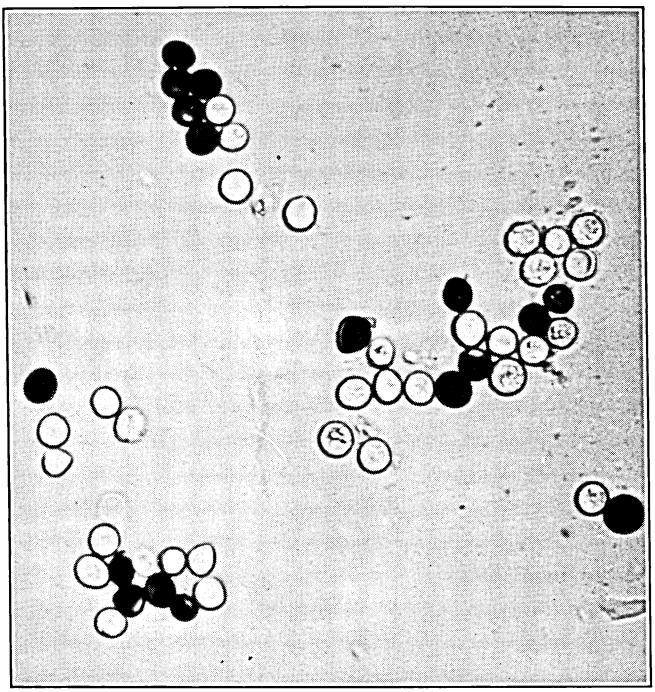


Plate 13. Photomicrograph Showing the Contrast in Color Between Chlamydospores of the Buff Type of Oat Smut and the Normal Dark Type

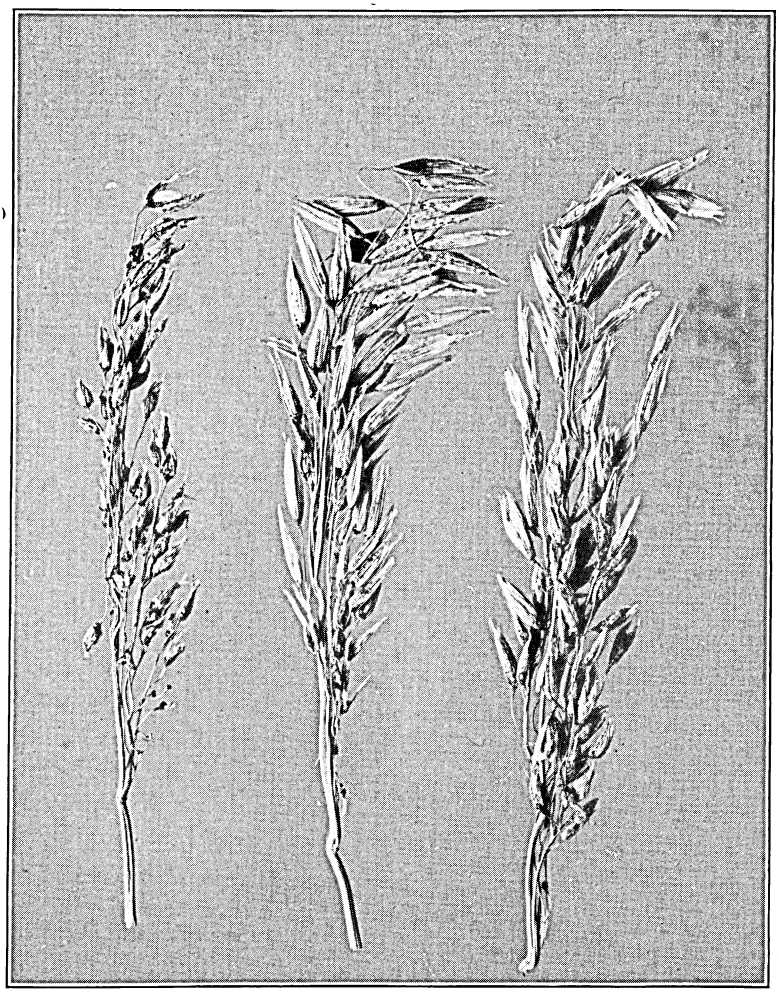


Plate 14. Loose, Intergrading, and Covered Types of Smut on Anthony Oats Produced by the Buff Smut Organism

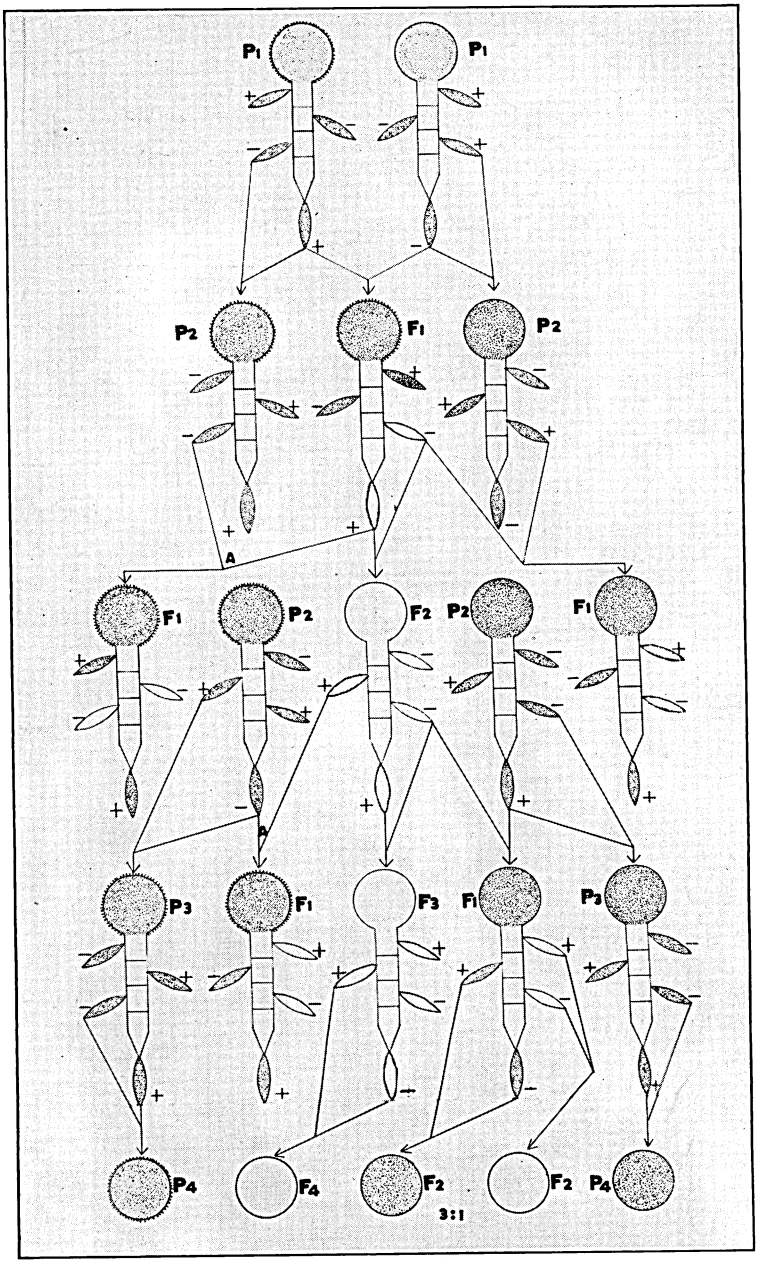


Plate 15a. Schematic Representation of the Inheritance of Chlamydospore Charateristics in Crosses Between *Ustilago avenae* and *U. levis*, and Showing Probable Sex Grouping (+ and —)

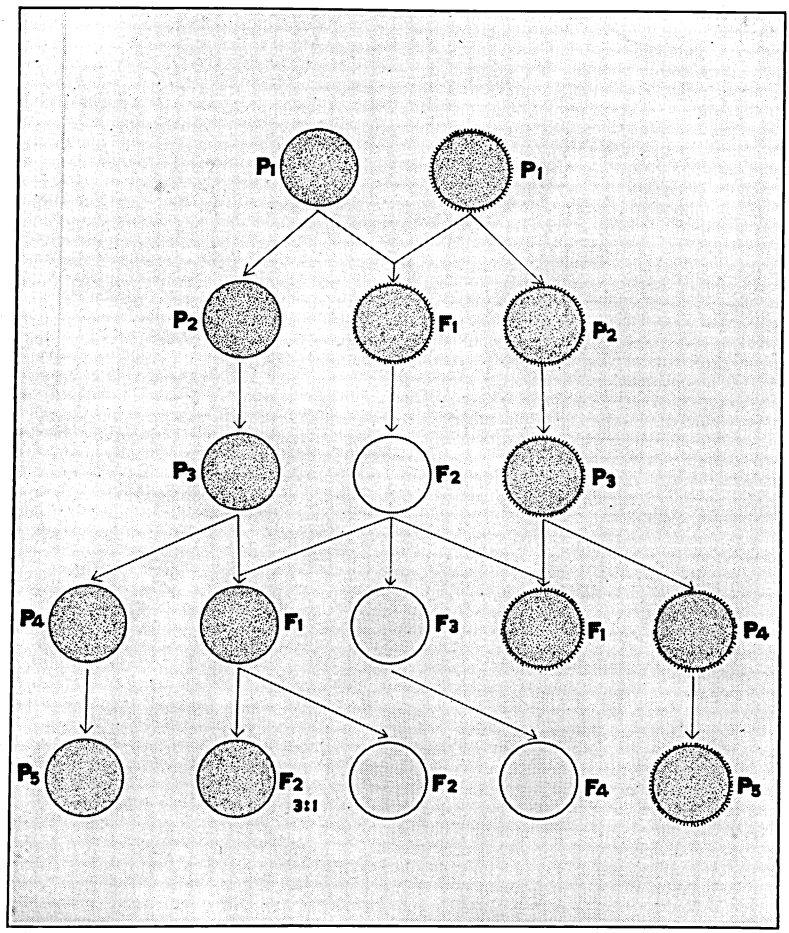


Plate 15b. Schematic Representation of the Inheritance of Chlamydospore Characteristics in Crosses Between *Ustilago avenae* and *U. levis*

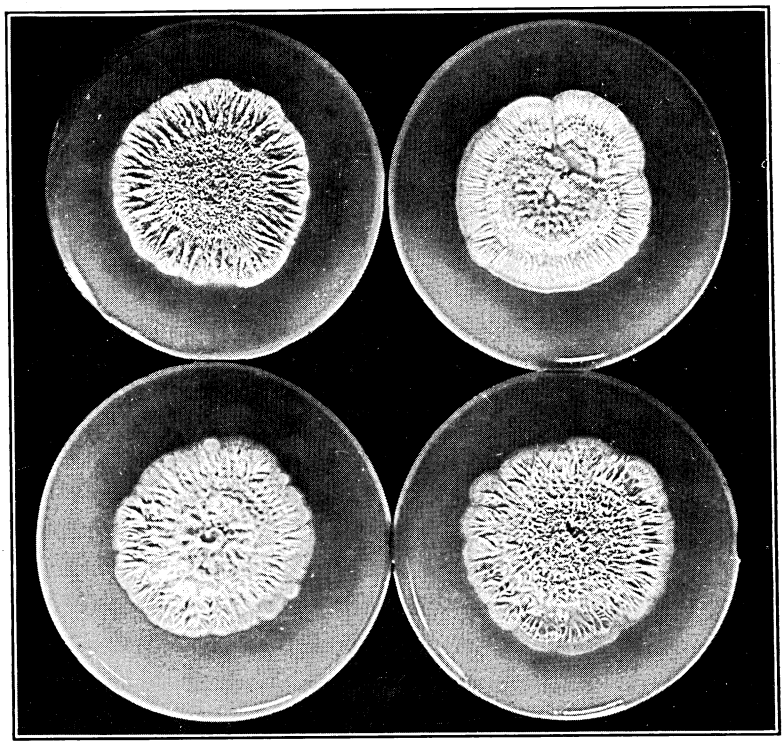


Plate 16. Four Monosporidial Lines of *Ustilago avenae* Obtained by Isolating Successive Sporidia from the Fourth Segment of the Promycelium of a Germinating Chlamydospore

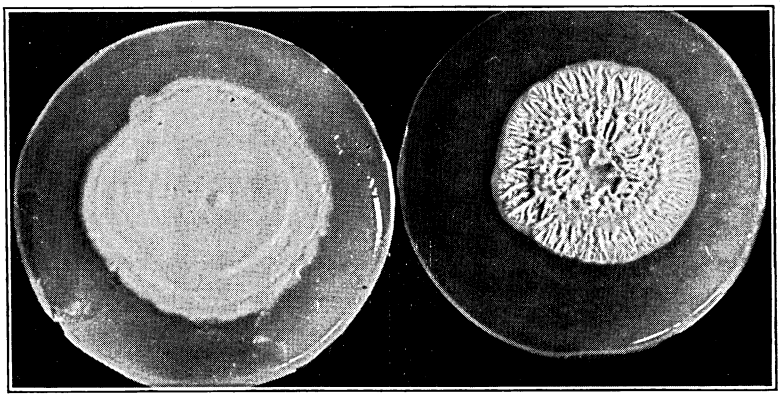


Plate 17. Two Monosporidial Lines of *Ustilago avenae* Obtained by Isolating Successive Sporidia from the Third Segment of the Promycelium of a Germinating Chlamydospore

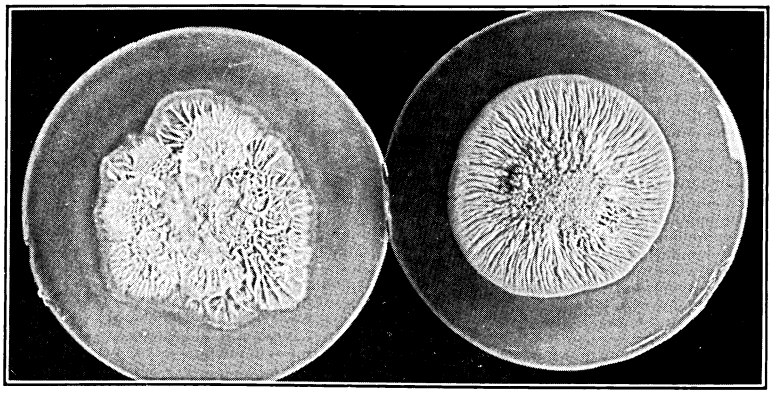


Plate 18. Two Monosporidial Lines of *Ustilago levis* Obtained by Isolating Successive Sporidia from the Second Segment of the Promycelium of a Germinating Chlamydospore

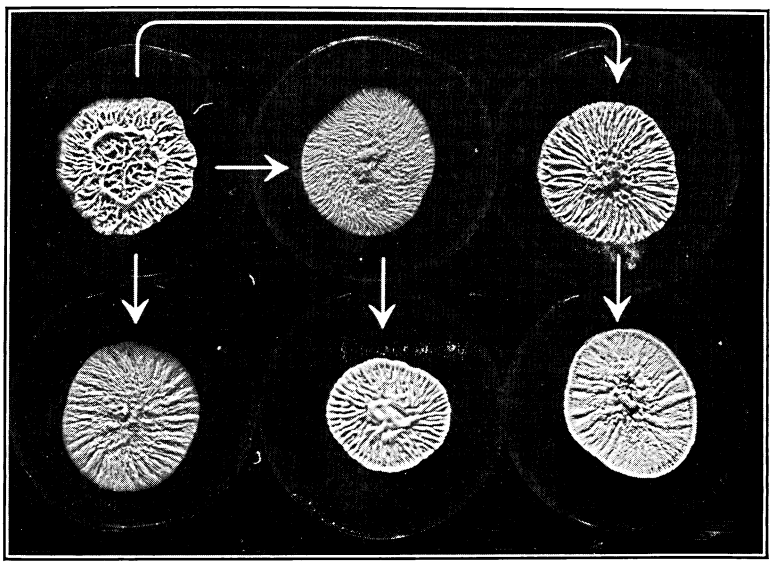


Plate 19. Cultural Variants of *Ustilago avenae* Obtained by Isolating from Sectors of a Monosporidial Line from Ireland
Upper left is the original; the others arose as sectors, as indicated by the arrows.

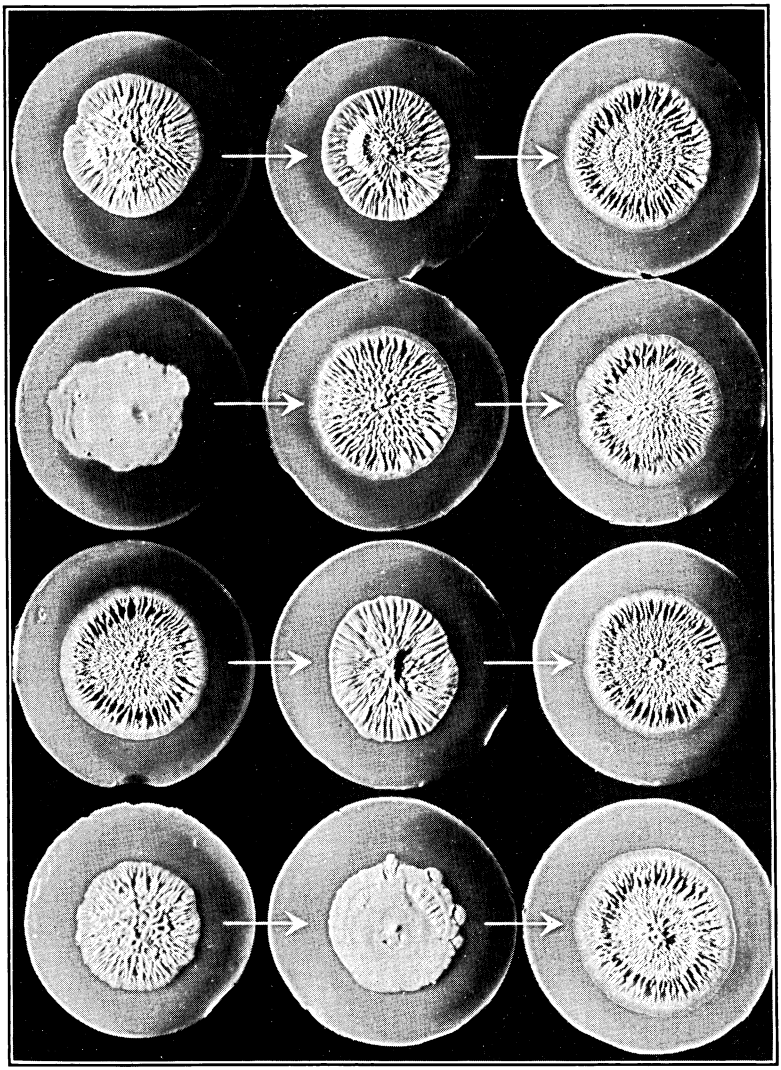


Plate 20. Four Monosporidial Lines of *Ustilago avenae* Derived from the Same Promycelium and Two Additional Lines of Each Derived by Isolating Sectors from the Original Lines

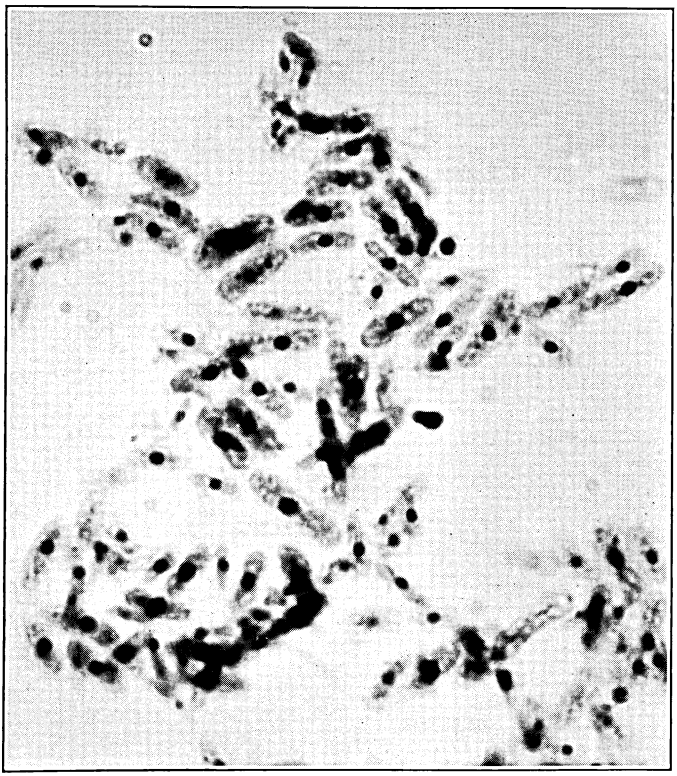


Plate 21. Photomicrograph of a Stained Preparation of *Ustilago avenae* Showing the Uninucleate Condition of the Sporidia