FURTHER STUDIES ON THE OAT SMUTS, WITH SPECIAL REFERENCE TO HYBRIDIZATION, CYTOLOGY, AND SEXUALITY

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INTRODUCTION

Hybridization in the oat smut fungi has been demonstrated by a number of investigators (2, 3, 7, 8, 10) and in some instances (5, 7, 10) pathogenically distinct races of Ustilago avenae (Pers.) Jens. and U. levis (Kell. and Sw.) Magn. have been produced artificially by this process. In 1931, the writer (3) described a buff smut of oats which appeared in hybrid material but which later was found to have arisen through mutation in U. levis (5). It was further shown that the buff smut fungus crosses readily with U. avenae and U. levis and that the buff character is inherited as a recessive (4, 6). Consequently, in such hybrids a buff F2 segregate is homozygous for this character. Therefore, by selecting a buff segregate on a variety immune from the buff parent and susceptible to the U. avenae or U. levis parent it is possible to obtain a race of the buff smut that possesses the pathogenicity of the other parent or of both parents. Such a hybrid race was reported by the writer (5) in 1936, and on this basis it appeared theoretically possible to produce, by hybridization, a race of the buff smut for every race of U. avenae and U. levis available. Accordingly, investigations were undertaken to determine the validity of this theory. Studies also have been made on nuclear behavior in the buff smut, on the inheritance of sorus type in two races of U. avenae, and on the process of sporidial fusion in all of the oat smuts. The results of these investigations are reported in this paper.

MATERIAL AND METHODS

The isolation of single sporidia and the determination of compatible combinations of monosporidial lines were accomplished by the methods previously described (4). Inoculations were made either by the method used formerly (3) or by the partial vacuum method described by Allison (1). Hybrid chlamydospores were obtained by inoculating Anthony (C. I. 2143) oats with paired monosporidial lines of Ustilago avenae and the buff smut and U. levis and the buff smut. Because of the high degree of sterility in sporidia from hybrid chlamydospores (3, 4), the F1 spores were used to inoculate differential varieties, and buff F2 segregates were selected from varieties immune from the buff parent. Inoculum of succeeding generations of the F2 buff selections was taken from the same varieties on which the F2 segregate appeared.

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2 Italic numbers in parentheses refer to Literature Cited, p. 240.

3 C. I. refers to accession number of Division of Cereal Crops and Diseases.
Two races of *Ustilago avenae* that produce distinctly different sorus types were used to study the heritability of this character. Hybrid spores were obtained in the manner described above, and since the sporidia from these F₁ spores will grow in culture, inoculations were made with combinations of monosporidial lines from the F₁ chlamydospores to obtain the F₂ population. Complete sets of four monosporidial lines were obtained from five F₁ chlamydospores; inoculations were made with the compatible combinations within each set, and one set was used in backcrosses to the parent lines.

The nuclei of the buff smut fungus in several stages of development were stained with Heidenhain’s iron-alum hematoxylin. The procedure described previously (4) was used, except that the material was destained with acid alcohol instead of iron-alum.

The process of sporidial fusion was studied by mating sporidia of opposite sex in pairs on plain water agar. The pairings were made with the aid of a Chambers’ micromanipulator and the sporidia in each pair were distinguished from each other by difference in size, i.e., small sporidia were selected from one line and mated with relatively large sporidia selected from the other line.

**RESULTS**

**PHYSIOLOGIC RACES OF BUFF SMUT**

Seven races of the buff smut have been identified on the basis of the differential reaction of certain oat varieties. One of these races arose through mutation, one represents a field collection, and five were produced by hybridization. The results of pathogenicity tests on which the separation of the races was based are presented in table 1. The origin and distinguishing characteristics of each race are as follows:

**Race 1.**—Mutant from *Ustilago levis* collected in McLeod County, Minn., in 1930. As shown in table 1, Canadian is highly susceptible to this race and 17 percent smut was produced on Richland.

**Race 2.**—An F₂ segregate on Gothland from a hybrid between buff race 1 and a Gothland race of *Ustilago avenae*. Differs from race 1 primarily by the susceptible reaction of Gothland to race 2.

**Race 3.**—Collected by Harland Stevens from a row of oats in a seed stock nursery grown for T. R. Stanton at Aberdeen, Idaho, in 1935, the seed of which was obtained from a Chicago, Ill., grain elevator. This race differs from race 1 by the susceptible reaction of Gothland and from race 2 by the susceptibility of Richland.

**Race 4.**—An F₂ segregate on Black Mesdag from a hybrid between buff race 1 and a Black Mesdag-Monarch race of *Ustilago levis*. This race is characterized by its capacity to infect Black Mesdag.

**Race 5.**—An F₂ segregate on Monarch from a hybrid between buff race 2 and a Black Mesdag-Monarch race of *Ustilago levis*. Its virulence on Gothland, Monarch, and Black Mesdag distinguishes this race from the others. It is notable that Monarch is more susceptible and Black Mesdag is less susceptible to this race than to the *U. levis* parent and that Gothland is less susceptible than it is to the buff parent (table 1).

**Race 6.**—An F₂ segregate on Trojan from a hybrid between buff race 1 and a Monarch-Richland race of *Ustilago levis*. This race is outstanding for its capacity to infect Alabama Red Rustproof, which is resistant to both parent races. It is definitely less virulent on Monarch than the *U. levis* parent and about equal in virulence to both parent races on Richland.

**Race 7.**—An F₂ segregate on Fulghum from a hybrid between buff race 1 and a Monarch-Fulghum race of *Ustilago levis*. This race is characterized by its virulence on Fulghum and Monarch and, to a slight degree, on Black Mesdag.
It is apparent from the foregoing results that new races of the buff smut may be produced at will by crossing any race of this fungus with races of *Ustilago avenae* and *U. levis*. A new race of the buff smut was obtained from every cross made between races of the buff smut and different races of these two species. In some cases the new races are similar in pathogenicity to the *U. avenae* or *U. levis* parent, while in others the virulence of the hybrid race is greater than that of either parent. For example, race 2 and its *U. avenae* parent both infect Gothland while all other varieties are resistant, and race 7 and its *U. levis* parent both infect Monarch, Black Mesdag, and Fulghum. Race 4, however, infects Black Mesdag only, whereas its *U. levis* parent infects Monarch in addition to Black Mesdag. On the other hand, race 5 infects all of the varieties (Gothland, Monarch, Black Mesdag) that the two parent races infect; and race 6, like its *U. levis* parent, infects Monarch and Richland but, unlike either parent, can infect Alabama Red Rustproof. Just how the infective capacity of race 6 for Alabama Red Rustproof arose cannot be explained by the data at hand.

Race 1 infects Canadian and, to some extent, Richland, and apparently it has the same pathogenicity as the *Ustilago levis* race from which it mutated. It is notable, however, that in earlier tests Monarch also was infected by this race (5). It would appear, therefore, that buff race 1 originally was heterozygous for pathogenicity and those biotypes capable of infecting Monarch were lost by selective elimination on another variety. Similar results have been obtained with race 2. Gothland and Monarch were infected by this race in the F<sub>3</sub> (5), whereas only Gothland was infected in the F<sub>8</sub> (table 1). Sampson and Western (9) have shown that there is a definite selective influence of the host on the relative stability of physiologic races of the oat smut fungi. They pointed out, however, that it would be theoretically possible for a heterozygous condition to persist through several chlamydoспоре generations, which would limit the efficiency of screening as a means of obtaining races pure for patho-

<table>
<thead>
<tr>
<th>Parent and hybrid races of oat smut</th>
<th>Percentage of smut in host testers—</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ustilago levis</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buff mutant race</td>
<td></td>
</tr>
<tr>
<td>U. <em>avenae</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buff mutant parent</td>
<td></td>
</tr>
<tr>
<td>Buff hybrid race</td>
<td></td>
</tr>
<tr>
<td>Buff field race</td>
<td></td>
</tr>
<tr>
<td>U. <em>levis</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buffer mutant parent</td>
<td></td>
</tr>
<tr>
<td>Buff hybrid race</td>
<td></td>
</tr>
<tr>
<td>U. <em>levis</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buffer mutant parent</td>
<td></td>
</tr>
<tr>
<td>Buffer hybrid race</td>
<td></td>
</tr>
<tr>
<td>U. <em>levis</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buffer mutant parent</td>
<td></td>
</tr>
<tr>
<td>Buffer hybrid race</td>
<td></td>
</tr>
<tr>
<td>U. <em>levis</em> parent</td>
<td></td>
</tr>
<tr>
<td>Buffer mutant parent</td>
<td></td>
</tr>
<tr>
<td>Buffer hybrid race</td>
<td></td>
</tr>
</tbody>
</table>
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genicity. Possibly this was the case with race 2, and the repeated selection of inoculum from Gothland was necessary to gradually eliminate the capacity for infecting Monarch. Little is known, however, regarding the stability of such hybrid races of the oat smuts. The majority of the races listed in table 1 (races 4, 5, 6, and 7) are in the $F_4$ generation, and if inoculum of future generations is taken from the same variety then the pathogenicity for certain other varieties may become lost. This would be highly probable in the case of a hybrid race that carries the pathogenic properties of both parents, such as race 5 (table 1). Race 5 was obtained by selecting a buff $F_2$ segregate on Monarch, and inoculum has been taken from the same variety in succeeding generations. Repeated selection of inoculum of this race from Monarch, however, might finally eliminate the Gotliland and Black Mesdag pathogenicity, or the selection of inoculum from Gothland might result in the loss of the Monarch and Black Mesdag virulence. On the other hand, if there were linkage of factors for virulence on these varieties, or if this virulence were controlled by a single factor, both of which considerations seem improbable in this case, then the reaction would remain constant in future generations regardless of the variety from which inoculum might be obtained, unless the linkage were to become broken or mutation were to occur.

Similar results might be expected with races 6 and 7, while race 4 probably will remain constant in virulence if the inoculum is always taken from Black Mesdag. Therefore, in the light of these considerations, it is possible that the host reaction of the buff smut races shown in table 1 will change to some extent by continuous propagation on specific varieties. Considering the pedigree of these races, however, it seems improbable that such changes as may occur will materially affect their differentiation.

It will be noted in table 1 that comparative tests with the parent or parents of race 3 were not made, the reason being that its pedigree is not known. This race was discovered in 1936 among a large number of collections of *Ustilago avenae* and *U. levis* obtained from the 1935 seed stock nursery at Aberdeen, Idaho. The row from which this buff smut specimen was obtained had been sown to seed collected in a grain elevator in Chicago, Ill. Eleven smutted panicles were in the collection, of which four were *U. avenae*, four *U. levis*, and three buff smut. No other specimens of buff smut were found in 1,877 smutted panicles examined, all of which came from the seed stock nursery mentioned above. This is the only record of the occurrence of the buff smut under natural conditions. Furthermore, the record would seem to preclude any possibility that this instance of natural occurrence of the buff smut was due to an escape from the experimental material, especially in view of the fact that the panicles smutted with race 3 are characteristically different from those of race 1, the original buff smut, or any of the hybrid races. Race 1 produces sori entirely covered by the outer glumes, while race 3 completely destroys the outer glumes, leaving the sori exposed. The other races, for the most part, partially destroy the outer glumes, thus producing a type of smutted panicle intermediate between the two extremes of races 1 and 3. The spores of the buff smut, like
those of *U. levis*, remain intact in the sorus and are not readily disseminated as in *U. avenae*. Since the original buff smut race arose by mutation (5) it seems probable that race 3 is the result of a recurring mutation in *U. levis* that took place under natural conditions. Consequently, the buff smut fungus might justifiably be recognized as a variety of *U. levis* or possibly even as a distinct species.

**CYTOLOGY**

The results of studies on nuclear behavior in the buff smut fungus indicate that this phenomenon is fundamentally the same as in *Ustilago avenae* and *U. levis*. The nuclear condition in the various stages of development that were observed is shown in figure 1. The mature chlamydospore contains a single diploid nucleus (fig. 1, A). Meiotic division of this nucleus accompanies spore germination and one nucleus becomes located in each cell of the promycelium (B). The promycelium occasionally has four cells but usually three, the spore functioning as the fourth cell. Mitotic division of the nuclei of the promycelium accompanies the budding of a sporidium from each cell and one nucleus passes into each sporidium, the other remaining in the promycelial cell from which other sporidia may bud (C). Each sporidium contains a single haploid nucleus, which divides when the sporidium buds and one nucleus passes into the daughter sporidium. Binucleate sporidia frequently are observed, in which case it is presumed that nuclear division preceded sporidial budding (D). When sporidia of opposite sex fuse, the nucleus from one passes through the copulation tube into the other, thus initiating the dikaryophase (E). Each pair of fused sporidia produces an infection hypha into which the paired nuclei pass (F). Presumably the binucleate condition persists throughout the parasitic stage, nuclear fusion occurring when the spores become mature. If this presumption is correct, then nuclear behavior in the buff smut is identical with that of *U. avenae* and *U. levis*.

**INHERITANCE OF SORUS TYPE**

Two types of smutted panicles produced by two races of *Ustilago avenae* were observed in one row of Victory (C. I. 560) oats in the physiologic race nursery at Pullman, Wash., in 1937. Most of the smutted panicles in this row had the usual brown powdery type of sorus. A few panicles, however, had a distinctly black indurate type of sorus, and the spores of this type were slightly darker in color and less prominently echinulate than those of the powdery type. In both types the glumes had been completely destroyed, as shown in figure 2. The results of inoculations with spores from the two types of sorus indicated that these characters were genetically distinct. Studies were undertaken, therefore, to determine the nature of their inheritance. Combinations of monosporidial lines were used for inoculum, and seed of Anthony (C. I. 2143) oats was inoculated and grown to maturity in the greenhouse for the F1 and in the field for the F2. The results of these studies are summarized in tables 2, 3, and 4.
Figure 1.—Camera-lucida drawings showing the nuclear condition in various stages of development of the buff smut fungus. *A*, Mature chlamydospores. *B*, Germinated chlamydospore prior to sporidial formation. *C*, Germinated chlamydospores with sporidia budding from the promycelial cells. *D*, Haploid uninucleate and binucleate sporidia. *E*, Pairs of conjugated sporidia showing nuclei in various stages of migration: *a*, Nucleus about to enter the fusion tube; *b*, nucleus passing through the tube; *c*, binucleate stage or dikaryophase resulting from the migration of the nucleus from one sporidium to the other; *d*, beginning the production of the infection hypha following initiation of the dikaryophase; *e*, binucleate stage in which nuclear migration was accompanied or followed by migration of the cytoplasm into the sporidium which contains the nuclei. *F*, Infection hyphae.
Studies on Oat Smuts with Reference to Hybridization

Figure 2.—Smutted panicles of Victory oats showing two types of sorus produced by two physiologic races of Ustilago avenae. A, Powdery type, which sheds spores readily. (Note spore masses that were shed when the panicles were tapped against the background.) B, Indurate type, which does not shed spores.

Table 2.—Type of sorus produced on Anthony oats by two races of Ustilago avenae and hybrids between them

<table>
<thead>
<tr>
<th>Parent sorus type</th>
<th>Chlamydo-spor No.</th>
<th>Number of sporidial combinations</th>
<th>Type of sorus produced by—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_1$</td>
</tr>
<tr>
<td>Powdery</td>
<td></td>
<td>4</td>
<td>Powdery</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2</td>
<td>Indurate</td>
</tr>
<tr>
<td></td>
<td>56X54</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57X55</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58X59</td>
<td>6</td>
<td>Powdery</td>
</tr>
<tr>
<td></td>
<td>55X60</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.—Segregation of factors for type of sorus in crosses between two physiologic races of *Ustilago avenae*

<table>
<thead>
<tr>
<th>Chlamydospore No. and genotype</th>
<th>Sporidium No.</th>
<th>Sex ¹</th>
<th>Gametes</th>
<th>Genotype</th>
<th>Ratio</th>
<th>Type of sorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>93 (Pp)</td>
<td>1</td>
<td>+</td>
<td>p</td>
<td>PP</td>
<td>1:2:1</td>
<td>Powdery.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>PP</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>1:2:1</td>
<td>Powdery.</td>
</tr>
<tr>
<td>94 (Pp)</td>
<td>1</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>4:0</td>
<td></td>
</tr>
<tr>
<td>95 (Pp)</td>
<td>1</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>1:2:1</td>
<td>Powdery.</td>
</tr>
<tr>
<td>96 (Pp)</td>
<td>1</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>4:0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>97 (Pp)</td>
<td>1</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>4:0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
<td></td>
</tr>
</tbody>
</table>

¹ P=powdery; p=indurate.

² Plus (+) and minus (−) signs indicate sporidin of opposite sex.

### Table 4.—Results obtained by backcrossing sporidia from a hybrid chlamydospore with sporidia from the parent chlamydospores to determine the heritability of sorus type

**USTILAGO AVENAE 94 (Pp) X U. AVENAE 56 (PP)**

<table>
<thead>
<tr>
<th>Sporidium</th>
<th>Sex</th>
<th>Gamete</th>
<th>Sporidium</th>
<th>Sex</th>
<th>Gamete</th>
<th>Genotype</th>
<th>Type of sorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>P</td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>PP</td>
<td>Powdery.</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>P</td>
<td>3</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>p</td>
<td>4</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>p</td>
<td>1</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>p</td>
<td>2</td>
<td>+</td>
<td>p</td>
<td>Pp</td>
<td>Do.</td>
</tr>
</tbody>
</table>

**USTILAGO AVENAE 94 (Pp) X U. AVENAE 54 (pp)**

<table>
<thead>
<tr>
<th>Sporidium</th>
<th>Sex</th>
<th>Gamete</th>
<th>Sporidium</th>
<th>Sex</th>
<th>Gamete</th>
<th>Genotype</th>
<th>Type of sorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>P</td>
<td>2</td>
<td>-</td>
<td>p</td>
<td>Pp</td>
<td>Powdery.</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>P</td>
<td>3</td>
<td>+</td>
<td>p</td>
<td>pp</td>
<td>Do.</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>p</td>
<td>4</td>
<td>+</td>
<td>p</td>
<td>pp</td>
<td>No infection.</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>p</td>
<td>1</td>
<td>-</td>
<td>p</td>
<td>pp</td>
<td>Do.</td>
</tr>
</tbody>
</table>

As shown in table 2, the 12 monosporidial combinations representing three chlamydospores from the powdery type of sorus produced that type of sorus in the F₁ and F₂, and the 12 monosporidial combi-
nations representing four chlamydospores from the indurate type produced that type of sorus. Apparently, therefore, the chlamydospores from which the monosporidial lines were obtained were homozygous for sorus type. The 28 combinations between monosporidial lines from the two types of sorus produced powdery sori in the F₁, indicating dominance of the factor for this type of sorus. In the F₂, there was segregation into powdery and indurate sorus types. The manner in which segregation of factors for the two types of sorus occurs is shown by the results from inoculations with crosses between monosporidial lines obtained from five F₁ chlamydospores (table 3), and by backcrossing the monosporidial lines from one F₁ spore with four monosporidial lines from one chlamydospore of each parent (table 4). As shown in table 3, there was independent segregation of factors for sex and sorus type in three of the chlamydospores (93, 95, 96), and the sorus type appeared in a ratio of 3 powdery to 1 indurate. The genotype ratio was found to be 1 : 2 : 1, as indicated. In chlamydospores 94 and 97, segregation of factors for sex and sorus type occurred in the same nuclear division and, therefore, all of the sori were powdery, the ratio being 4 : 0, as shown. Theoretically, these F₂ chlamydospores are heterozygous and should produce both types of sorus in a 3 : 1 ratio in the F₃, if segregation of factors for sex and sorus type occurs independently. In the backcrosses with the powdery parent (Ustilago avenae 56, table 4) all of the combinations produced powdery sori, as expected, since this character is dominant. The spores of half of these backcrosses were determined to be homozygous for the powdery sorus and the other half heterozygous. In the backcrosses with the indurate parent, four of the crosses produced powdery sori the spores of which were heterozygous, two crosses produced indurate sori the spores of which were homozygous, and two crosses failed to infect. Presumably the two crosses that failed to infect would have produced the indurate type of sorus if infection had occurred (table 4), in which case the spores would have been homozygous. All of the indurate sori were black, in contrast to the dark brown of the powdery sorus. This color contrast probably is due to the slightly darker spores in conjunction with color intensification caused by a more effective elimination of air spaces in the indurate sorus.

It is apparent from these studies that the factor for powdery sorus type is dominant over the factor for indurate sorus type and that the segregation and recombination of factors in the F₂ occurs in a 1 : 2 : 1 ratio. Chlamydospore markings and color are known to be inherited in a similar manner (6).

SPORIDIAL FUSIONS

In a previous report (4) it was shown that in the fusion process between any two sporidia of Ustilago avenae and U. levis one sporidium is active while the other appears to be passive. Since the sporidia normally are morphologically indistinguishable it was not determined whether the active sporidia were of the same sex and the passive sporidia of the opposite sex. Recently, however, it was found possible to modify the morphology of sporidia to such an extent that those of one line can readily be distinguished from those of another line. As shown in figure 3, sporidia that bud on potato-dextrose agar are larger and more uniform in size and shape (fig. 3, A, D) than those
that bud on plain agar (fig. 3, B, E). Furthermore, sporidia from plain agar usually have no food vacuoles, while those from potato-dextrose agar may have one to several, the usual number being two.

Thus, for example, by pairing the sporidia of sex A grown on potato-dextrose agar with the sporidia of sex B grown on plain agar, or the sporidia of sex A grown on plain agar with the sporidia of sex B grown
on potato-dextrose agar, it is possible to determine which sporidia are active and which are passive in the fusion process.

Such pairings, which may be made with the aid of a micromanipulator, are shown in figure 3, C, F, and the ease with which the sporidia are distinguished from each other is obvious. By this means observations were made on pairs of plus (+) and minus (−) sporidia of Ustilago avenae, U. levis, and the buff smut. In 49 pairs of U. avenae sporidia, the plus (+) sporidia, taken from plain agar, were active and the minus (−) sporidia, taken from potato-dextrose agar, were passive, while in 39 other pairs the minus (−) sporidia, taken from plain agar, were active and the plus (+) sporidia, taken from potato-dextrose agar, were passive. In 10 pairs, however, the plus (+) sporidia, taken from potato-dextrose agar or plain agar, were active while the minus (−) sporidia, taken from either medium, were passive. Thus, in 88 pairs the sporidia taken from plain agar were active and the sporidia taken from potato-dextrose agar were passive, regardless of sex, while in 10 pairs the plus (+) sporidia were active and the minus (−) sporidia were passive, regardless of the medium from which they were taken. In 93 pairs of U. levis sporidia and 80 pairs of buff smut sporidia the sporidia taken from plain agar were active and those taken from potato-dextrose agar were passive, regardless of sex. Therefore, it appears from these observations that apparent active or passive participation in the fusion process is governed primarily by the "physiological" condition of the sporidia and not by their sex. Obviously, those sporidia which are grown on plain agar are in a "starved" condition and apparently respond more rapidly to the fusion stimulus than those which are grown on potato-dextrose agar and have an abundance of reserve nutrients. The 10 pairs of U. avenae sporidia mentioned above were an exception to the general rule.

The increased tendency for sporidia of the smut fungi to fuse when placed under conditions of low nutrients is a generally recognized fact. Consequently, the mating of sporidia from a low-nutrient medium with sporidia from a high-nutrient medium might be considered an unfair test of their active and passive reactions. For this reason it seemed desirable to mate sporidia of opposite sex that had been grown on the same medium. Accordingly, observations were made on pairs of sporidia in which the larger sporidium of each pair represented one sex and the smaller sporidium the other sex, both sporidia being taken from potato-dextrose agar. Twenty pairs of buff smut sporidia were observed and the plus (+) sporidium was active in 9 pairs, the minus (−) sporidium was active in 9 pairs, and both sporidia were active in 2 pairs. Similar results were obtained with 20 pairs of Ustilago avenae sporidia, but no observations were made on U. levis. These results further indicate that sporidia of opposite sex in the oat smut fungi may appear active or passive in the fusion process, depending, at least in part, upon their "physiological" condition.

In the course of the studies on sporidial fusions, two sizes of sporidia were observed in one monosporidial line of the buff smut on plain agar. This size difference can be seen in figure 3, E, where a colony of the larger sporidia is shown adjacent to and coalesced with a colony of the smaller sporidia. These two colonies developed from single
sporidia, and since both sizes of sporidia were present in a mono-
sporidial line it is possible that one type arose from the other through
mutation.

SUMMARY

Seven physiologic races of the buff smut of oats are described, which
either arose through mutation or were produced by hybridization
with *Ustilago avenae* or *U. levis*.

Mature chlamydospores of the buff smut contain a single, diploid
nucleus. Reduction division accompanies spore germination and
each cell of the promycelium contains a single haploid nucleus, as do
the sporidia which bud from the promycelium. When two sporidia
fuse, the nucleus from one passes into the other and both nuclei pass
into the infection hypha. The nuclear behavior of the fungus in the
host has not been studied, but presumably the dikaryophase persists
throughout the parasitic stage and the nuclei fuse at the time of
chlamydospore formation.

The factor for powdery sorus type is dominant over the factor for
indurate sorus type. Segregation and recombination of factors for
these characters occurs on a simple 3:1 ratio basis.

Active and passive participation of sporidia of the oat smuts in
the fusion process appears to be dependent, at least in part, upon the
"physical" condition of the sporidia rather than upon their sex. For
example, sporidia that bud on plain agar respond more rapidly to the
fusion stimulus than sporidia that bud on potato-dextrose agar.

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