STUDIES ON THE RELATION OF CERTAIN MORPHOLOGICAL CHARACTERS OF THE HOST AND FUNGUS TO THE IDENTIFICATION OF THE LOOSE AND COVERED SMUTS OF OATS

by

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INTRODUCTION

Oat smut has been known since the time of Pliny. At that time, however, it was confused with other cereal smuts, especially with the stinking smut of wheat. Although occasional references to the smut of oats occur in early writings, it was not recognized as an organism until the time of Linnaeus, but was considered merely as an abnormal

condition of the host. It was in 1889 that Jensen (16) recognized oat smut as a distinct species distinguishable from the smuts on wheat and barley. He called it Ustilago avenae. Before this time observers had not discovered any differences in these smuts except for the relative abundance on different host crops.

In 1882 a specimen of oat smut from Shelburne, New Hampshire, was found to have spores that were entirely smooth-walled with granular cell contents. These characters were thought to be due to imperfect maturation of the spores and the matter was given no further consideration.

In 1889 Kellerman and Swingle (18) found a number of smutted panicles in Kansas having similar smooth spores. They gave this newly discovered form of smut varietal rank, calling it Ustilago avenae var. levis. It was later changed to species rank by Magnus (21). The appearance of the panicle was found to be somewhat different from that infected by U. avenae, due to a greater firmness and persistence of an outer membrane and a grayish color produced by the dark spores showing through the light membrane of the panicle. In the more recent literature the smuts produced by U. avenae and U. levis are commonly called "loose" and "covered" smuts respectively.

Since the publication of Kellerman and Swingle's paper, writers have, almost without exception, described the two species as being entirely different in appearance and easily distinguishable macroscopically. However, many infected panicles are intermediate in appearance, and investigators familiar with oat smut find they cannot determine the species with any degree of accuracy except by chlamydospore examination. This situation has led to considerable uncertainty and confusion. For this reason it was thought advisable to examine a large number of smutted panicles and study the spores to determine whether most of the distinctions between loose and covered smuts are untrue or misleading, or whether there is, after all, some definite character by which the two species can be distinguished without spore examination.

Numerous collections of smut containing U. levis and at least two physiologic forms of U. avenae were carefully examined to ascertain any gross morphological differences in panicle characters between the physiologic forms of U. avenae and any differences within the same physiologic form on different oat varieties that would approach the difference between the two species and add to the difficulty in distinguishing between them.

Rodenhiser (29) found that physiologic forms of oat smut could be distinguished by their appearance on certain culture media. It was, therefore, thought that similar experiments with the physiologic forms grown at the Kansas Agricultural Experiment Station might throw additional light on the problem of distinguishing between forms and species of oat smut.

Plant decoction media that have been used almost exclusively for such studies are unsatisfactory because of their variability. This is due to such factors as variety, conditions of growth, and duration of storage of the plant materials used. It was decided, therefore, to attempt to grow cultures on synthetic media. These are more satisfactory as they can be prepared with greater precision and with exactly the same nutrient balance time after time in the same laboratory or by other investigators.

REVIEW OF LITERATURE

Kellerman and Swingle (18) described U. avenae (Pers.) Jens. as converting practically all of the spikelets of the panicle into a loose powdery mass of spores. It often almost completely destroys the normal tissue, leaving only a black mass of spores penetrated by shreds of tissue. More often, however, the glumes retain more or less of their

normal appearance, the smut destroying them at the base and breaking through in isolated pustules toward the tips. In smutted spikelets, the lesions are at first covered by a thin membrane which soon disappears. The spores are rapidly carried away by wind and rain, until sometimes nothing remains but the bare rachis. Occasionally, a smutted panicle has normal outer glumes and the presence of smut can hardly be detected, but such instances are infrequent. The smutted panicles usually grow up free from the leaves as the healthy ones do, but occasionally one remains partially enclosed by the sheath of the upper leaf. The smut fungus also frequently attacks the foliar tissues producing long black smut lesions on the leaves and leaf sheaths. Sometimes a smutted plant may be detected before flowering time by its stouter culms. Usually all culms of a plant are diseased. Usually also, all spikelets of a panicle are affected. When this is not the case, it is always the lower ones that are diseased, indicating that the infection comes from below.

According to Kellerman and Swingle (18) U. levis (K. & Sw.) Magn. is distinguished by the more firm and persistent outside membrane of the glumes and by a grayish color probably due to the dark spores showing through the

light membrane. The two forms are often hard to distinguish.

Some writers including Bolley (2), Tubeuf (37), McAlpine (22) and Butler (3) have given descriptions of U. avenae much like that of Kellerman and Swingle but have not described U. levis.

Kellerman and Swingle (19) described "hidden smut" produced by U. levis, in which the glumes are unaffected and apparently enclosing normal grains. It can usually be detected by the following obscure characters: (1) usually a greener color without the yellowish tinge of sound ripening panicles, (2) the tips of the outer glumes are usually bleached, and (3) some of the grains, especially the lower ones, are stunted. Swingle (36) and Clinton (5) also mention "hidden smut" but in every case it is stated that all U. levis is not "hidden smut."

Duggar (10) describes U. levis as always being hidden. He states that in the case of U. avenae the cells of the host are absorbed until the whole ovary and enclosing glumes are converted into a mass of sooty spores but he gives an illustration which corresponds very closely to other writers' descriptions and illustrations of U. levis.

Clinton (5), Stevens and Hall (35), Stevens (34), and Güssow and Conners (13) described U. avenae very much as Kellerman and Swingle and others had done, but distinguished U. levis only by the less complete destruction of the spikelet, believing that only the inner and basal parts of the glumes are affected. They made no mention of the grayish color mentioned by Kellerman and Swingle. Stevens (34) stated, however, that U. levis is probably more common than records show as it is difficult to distinguish from U. avenae.

Gage (12), in his infection and life history studies, found it necessary to examine spores microscopically to make certain of their rough-walled character since the so-called "loose" character of the lesions caused by U. avenae was not consistent, gradations being found that correspond more closely to the covered character of the U. levis lesion.

There is little or no disagreement among writers in the description of the spores and none of them have found any difference in size or shape between the two species. Kellerman and Swingle (18) described U. avenae spores as dark, dusky brownish, no olivaceous tinge; mostly oval but

may be subglobose, elliptical, irregular or deformed. The size varies. They are 5-11 x $4\frac{1}{2}$-7μ, mostly 6-9 x 5-7μ. The wall has two layers, an outer deeply colored epispore and a paler endospore. The spore is lighter colored on one side and germinates from this region. The epispore is covered with minute elevations or warts. There is a small area on the light side that is free from spines, most likely where the germ tube arises. The contents of the spore are homogeneous and only rarely granular. The spores of U. levis are somewhat darker brown than those of U. avenae. They are similar in size and shape and also have a double wall and are lighter on one side. They are distinguished by having an epispore that is smooth, at least not spiny or punctate. Sometimes thicker portions appear but they are slight and not like the spines of U. avenae. The contents are frequently granular or guttate.

Others, including Clinton (5) and Güssow and Conners (13) have described them more briefly somewhat as follows: U. avenae--subspherical to spherical, sometimes more elongate, 5-9μ in diameter, lighter colored on one side, minutely echinulate. U. levis--similar but perhaps more uniform in size and shape, darker in color, wall smooth.

Rodenhiser (29) mentioned finding all gradations of echinulate markings from practically smooth to highly echinulate in chlamydospores from a single panicle supposedly infected with U. avenae.

In respect to germination and cultural studies, several investigators reported the best germination at room temperature, Herzberg (15) and Hecke (14) reporting 22°-30° C., Tubeuf (38) 20°-21° C., Jones (17) 21.7° C., and Bartholomew and Jones (1) 15°-28° C. for U. avenae and Novopoknovsky and Skaskin (23) 20°-25° C. for U. avenae, U. hordei, U. nuda, and U. tritici. On the other hand, Sampson (31) and others found that the percentage of germination decreased steadily as the temperature was raised above 15° C.

Bartholomew and Jones (1) found 20° C. to be the optimum temperature for growth of U. avenae on potato dextrose agar. Rodenhiser (29) found that room temperature was satisfactory for growing and distinguishing a number of physiologic forms of U. avenae and U. levis on potato dextrose agar. He found that none of the forms could be distinguished by rate of growth at any temperature between 10° and 35° C. Dickinson (9) found that room temperature was satisfactory for growth of U. levis and U. hordei and that no variations in colony type occurred within this temperature range.

Little work seems to have been conducted with single chlamydospore or single sporidium cultures. Rodenhiser (29) makes no mention of using anything but multispore cultures. Bartholomew and Jones (1) found the single spore method of culturing the organism impracticable because of the minute size of the spores, their tendency to cling together, and their poor germination, even when incubated under optimum conditions.

Dickinson (7, 8, 9) isolated single spores of U. levis and U. hordei, even including single sporidia from known positions on the promycelium by means of the Dickinson isolator (6).

Rodenhiser (29) used two per cent potato dextrose agar after making preliminary tests with a number of other media. Dickinson (9) used a synthetic medium that will be referred to later.

Physiologic forms of some fungi have been known for a number of years. Two physiologic forms of oat smut were discovered by Reed (25) in 1924, and by Sampson (30) by means of infection studies. A number of other forms have been discovered by the same method and reported by Reed (26, 27) and Sampson (32). Christensen and Stakman (4) described 15 physiologic forms of U. zeae distinguishable

by such cultural characters as rate of growth, color, topography, surface, zonation, conidial production, and margin. Rodenhiser (29), by means of similar characters, was able to distinguish 18 forms of U. avenae and 5 of U. levis besides several forms of U. tritici, U. nuda and U. hordei. Ficke and Johnston (11) have made similar studies with Sphacelotheca sorghi and Kienholz and Heald (20) with Tilletia levis and T. tritici. Physiologic forms are common, therefore, in the smuts.

MATERIALS AND METHODS

A Study of Spore Markings and their Relation to Panicle Characters

Collections in the Herbarium of the Department of Botany and Plant Pathology. A number of specimens in the mycological herbarium of the Department of Botany and Plant Pathology of the Kansas State College of Agriculture and Applied Sciences were selected at random. These were studied to learn the typical panicle characters and spore markings for the two species of oat smut. It should be noted that whenever the word "spore" is used in these studies, it refers to the

chlamydospore. Many of the spores were measured and were found to fall within the limits specified by previous investigators. No difference in dimensions was apparent between spores of the two species. The measurements, therefore, are not recorded in Table I.

It was found necessary to use the oil immersion lens to see with any degree of certainty even the most distinct echinulations on spores of U. avenae. For that reason this magnification was used in this and all the succeeding microscopic examinations herein referred to.

Spores were taken from at least two places in a panicle selected as typical of each collection. Three mounts were made from panicles that varied in appearance from base to tip. Spores from different parts of the same panicle were the same in every case and only one record, therefore, is tabulated for each panicle. In collection No. 12 it was necessary to study spores from two different panicles since the panicles in the collection were of two types in general appearance. Results are found in Table I under experimental results.

Collections from Experiment Stations in Several States. With the panicle and spore characters of U. avenae and U.

levis in mind, as the result of the preliminary studies with the herbarium specimens, it seemed desirable to study the panicle characters and spore markings of large numbers of unidentified specimens with an attempt to determine whether there was any correlation between the two types of characters.

It was thought that specimens from different parts of the United States would be interesting material upon which to work. Besides merely furnishing material for the studies, such collections would indicate whether the same species differed materially under different climatic conditions or upon different varieties of oats as grown in various parts of the United States. The herbarium specimens were from several states also and even from foreign countries, but some parts of the United States were not represented. Besides, most of the specimens were very old and such dry, crushed material would not reveal the minor differences that might be found in fresh material.

To obtain the material desired, men at agricultural experiment stations in several different states were asked to send what they thought to be loose and covered smut of oats without making spore examinations. They were also

asked which species was more prevalent in their localities. Generous collections were supplied by almost everyone to whom the request was made. Eight answers were received to the question concerning the prevalence of the two species.

The writer had previously studied part of the collections from the Kansas Agricultural Experiment Station discussed later, and from these and the herbarium specimens referred to above, had reached some conclusions on how to identify U. avenae and U. levis by panicle characters. Thus, when the specimens from the several states arrived, the writer indicated which species she thought each specimen to be, merely from macroscopic examination.

The collections were studied in a manner similar to that used for the herbarium specimens. A typical panicle from each collection was described. In case there was an appreciable variation between panicles in the same collection, two or more were listed separately and described. Spores from near the base and near the apex of every panicle described were examined microscopically and spore markings recorded in Table II, page 43.

Collections Made by C. O. Johnston and L. E. Melchers at the Kansas Agricultural Experiment Station in 1925. Results of the studies discussed above as shown in Table II show that in a majority of cases the species was named correctly without spore examination by the investigator sending the material, and in almost every case was named correctly by the writer. This did not, by any means, justify answering in the affirmative the question of whether one could identify the two species without spore examination. Anyone upon being asked to send collections of the two species would naturally send a typical collection of each species if they were available in his locality and that is undoubtedly what was done. If material was studied which included many intermediate and non-typical specimens the percentage of specimens named correctly without spore examination would be likely to be very much lower. There would also be the possibility of characters being revealed in the intermediate forms that were not present in the typical specimens.

Material containing such intermediate smutted panicles was available in a collection made by C. O. Johnston and L. E. Melchers in 1925 from the seed treatment plots at the Kansas Agricultural Experiment Station. Having realized for some time that they were probably not successful in

correctly determining the species of oat smut in the field, they collected a large number of smutted panicles of varying appearances, to be used in such a study as this, when time permitted. The ones that they thought were typical loose smut consisted of 50 panicles and were labeled "loose smut." In these the glumes were almost wholly replaced by the blackish spore masses, in most cases powdery. Ninety-nine panicles that were not powdery and the glumes of which were quite largely not replaced by spore masses, producing partly hidden smut, they labeled "covered smut." The 49 panicles that were intermediate between these two groups in appearance were labeled by them "intermediate." They did not attempt to classify the last group as to the smut which they thought was present.

Fifteen panicles were selected at random by the writer from each of the groups marked "loose smut" and "intermediate" respectively. These were examined for panicle and spore characters as in the previous studies. Spores were examined from at least two places in each panicle. The writer's opinion as to the species of smut present in each panicle was recorded in every case before the microscopic examination was made. After 16 panicles selected at random from the "covered" group were studied in a like manner, and all found to have echinulated spores, eight other specimens

that most nearly resembled the writer's conception of covered smut from the remainder of the group were studied, making a total of 24 from this group. The results of all these determinations are found in Table IV, page 53 , and are discussed under experimental results.

Collections from the Oat Smut Nursery of the Kansas Agricultural Experiment Station. Although there seemed to be enough evidence from the studies made by the writer to justify drawing conclusions, it was decided to make additional studies before doing so. It was hoped that more panicles infected with U. levis would be found for study, since almost all of those examined were found to be infected with U. avenae.

Furthermore, it was desired to ascertain if possible just what the situation, with regard to the presence of the two species of smut, really is in the southern Great Plains. Until very recently Kanota, a selection from Fulghum, and other closely related varieties of oats of the red oats (Avena byzantina) type, have exhibited marked resistance to smut in the southern Great Plains. It has been the general impression, from casual observations made by various investigators that the species involved was U. avenae. In recent years, however, Kanota and Fulghum as grown in Texas, Oklahoma, and southern Kansas have shown

increasingly large amounts of smut. Infections of 40 per cent or more have been observed in recent years. The smut on these varieties proved to be a physiologic form of smut apparently originating somewhere in the south and gradually spreading northward. This raised the question as to the species of the new form, and whether it could be distinguished from other forms or species by any gross morphological characters. It was, therefore, decided to study several collections of oat smut made in the southern Great Plains and compare them for panicle and spore characters.

Excellent material was available for the study in the 1929 oat smut nursery of the Kansas Agricultural Experiment Station. Its origin was as follows: Fifteen collections of smut, secured at various points in Texas, Oklahoma, and Kansas over a period of five years, were grown on several varieties of oats at Manhattan. These collections were grown primarily to determine whether or not they differed in their host preferences. The data secured indicated that there were at least three forms, differing sharply in that respect. It was not known whether they were different physiologic forms of the same species or whether both species were included. Examination to determine the species was therefore necessary.

Each collection of smut was used to inoculate a uniform set of 13 varieties of oats. The varieties used were as follows:

Variety	Accession No.
Kanota	Ks. No. 5179
Red Texas	Ks. No. 5316
Burt	Ks. No. 5219
Richland	Ks. No. 5209
Markton	Ks. No. 6113
Fulghum	Ks. No. 5217
Red Rust Proof	C.I.No. 1355
Frazier	C.I.No. 2381
Navarro	C.I. No. 966
Fulghum	C.I. No. 708
Kanota	C.I. No. 839
Nortex	Tex.No. 9235
Fulghum (King)	C.I. No. 850

The smut collections were as follows:

Collection No.	Row No.	Source	Year	Variety of oats
1	1-13	Manhattan, Kans.	1927	
2	14-26	Stillwater, Okla.	1928	
3	27-38	Manhattan, Kans.	1928	
4	39-50	Rest, Kans.	1927	Red Texas
5	51-62	Columbus, Kans.	1928	White

6	63-74	Belleville, Kans.	1928	unknown
7	75-86	Blue Rapids, Kans.	1928	unknown
8	87-98	Roosevelt, Okla.	1928	Red Texas
9	99-110	Stillwater, Okla.	1927	Kanota
10	111-122	Denton, Tex.	1925	Fulghum
11	123-134	Denton, Tex.	1925	Kanota
12	135-146	Denton, Tex.	1928	Kanota*
13	147-158	Denton, Tex.	1928	Fulghum
14	159-170	Concordia, Kans.	1928	unknown
15	171-182	Belleville, Kans.	1928	unknown

When the smut was mature the entire crop was harvested and the smutted panicles from each row stored in a separate paper sack. A typical panicle was selected for study from each row and several panicles in case of variation within a row. The forceps were flamed between every selection to avoid contamination between rows.

Panicle and spore studies were made as already mentioned from every row containing smut in collections Nos. 1, 2, 3, 7, and 13. Measurements were taken of a number of spores, but nothing of special interest was observed.

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*From Manhattan.

Inoculum made from the other collections was studied for spore wall markings and panicles typical of each collection were described. Where the panicle characters of any row differed greatly from the typical, or if a row contained two or more differing types of panicles, note was made of this and the spores studied separately.

When the 1930 crop became available, observations on it were added to these studies. An attempt was made to make observations in the field but this was found to be very difficult and it was decided to wait until the crop was harvested.

The oat varieties were not identical with those used the previous year but were as follows:

Variety	Accession No.
Kanota	Ks. 5179
Red Texas	Wash. 768
Richland	Iowa 105
Markton	Ks. 6113
Fulghum	Ks. 5217
Red Rust Proof	C.I. 1355
Frazier	C.I. 2381
Navarro	C.I. 966
Nortex	Tex. 9235

The smut collections used were the same as those used in

1929 with two exceptions. Number 6 was a collection taken at Paola, Kansas, in 1929 in place of the Belleville, Kansas, collection used the previous year, and No. 12 was a collection from Kinsley, Kansas, 1929, substituted for the Denton, Texas, collection on Kanota oats.

It was not considered necessary to make spore examinations of these collections, but panicle characters were studied much more in detail. Observations were made upon the entire smutted crop instead of single panicles from each row as was done the previous year. All of the collections, with the exception of collection No. 15 were laid out side by side on tables and careful comparisons were made between different oat varieties infected with the same smut collection and between different smut collections on the same variety of oats. The information gained was recorded under the following headings; percentage of glumes present, amount of shredding of the glumes, shedding of spores, color of the glumes, and percentage of partially smutted heads.

As previously stated, the chief purpose of all these studies of spores and panicles was to determine whether there was any correlation between panicle characters and spore markings. Additional questions that might be answered by this more detailed panicle study were (1) whether the

same smut may produce such varied symptoms on two oat varieties as to give the appearance of being two different forms or species, and (2) whether upon careful examination one can detect differences between the physiologic forms by means of panicle characters.

Cultural Studies

The oat smut collections grown at the Kansas Agricultural Experiment Station in 1930 for infection studies were used for the cultural studies. The collection originally from Manhattan, Kansas, including rows 1-9, and the Manhattan, Kansas, collection including rows 19-27 were the only collections of covered smut present. It was not known whether these were two separate physiologic forms since the difference in percentage of infection on different oat varieties was slight, but it was decided to use both of them. That of rows 1-9 was designated C_1 and the other C_2.

A number of the collections of loose smut characteristically produced a high percentage of infection on Kanota oats and a low percentage on Richland. The collection from Roosevelt, Oklahoma, comprising rows 64-72 was selected to represent this form and was called L_1. Several other collections produced a low percentage of infection on Kanota

and a high percentage on Richland. The collection from Columbus, Kansas, comprising rows 37-45 was selected to represent this form and was called L_2. The collection from Paola, Kansas, produced a low percentage of infection on all the varieties of oats that were attacked. This was selected as a possible third form of loose smut and was designated as L_3. It was not known positively from infection studies that it was a physiologic form as it had been under observation for only one year.

The first problem that presented itself was a method of securing single chlamydospores from which to grow pure line cultures. Most other workers had used multispore cultures and this could be done again but was much more subject to error and confusion because of the possibility of a panicle being infected with two or more physiologic forms.

The first method attempted was that of transferring droplets of a liquid medium containing germinating spores by means of a micropipette. This involved using a liquid medium that would induce satisfactory germination. It was not satisfactory to isolate ungerminated spores because these smut spores have a low percentage of germination. A sugar solution was tried as this had been reported as satisfactory by Sampson (31). It was found, however, that normal promycelia and sporidia were not produced but rather de-

formed appearing germ tubes. An oat decoction made by soaking 500 gm. of oats in 1000 cc. of water for three or four hours and filtering or decanting was found to be very satisfactory for germination.

Uncontaminated spores were obtained by dipping a spikelet of smutted oats into a 1-1000 solution of $HgCl_2$, and then taking spores from the interior of the smut mass.

Spores were germinated at room temperature for 24 hours in drops of oat decoction in Van Tieghem cells. Repeated attempts were made to isolate single germinated chlamydospores from these drops but with no success. Some of the reasons for failure were the great tendency for the spores to cling together, the low percentage of germination when spores are single, and the smallness of the spores, which resulted in confusing ungerminated spores with small particles of foreign matter.

The next method attempted was the usual dilution method. Spores were transferred from a smut ball to a few cc. of sterile distilled water in a test tube. One or more loopfuls were transferred to a tube of melted and partly cooled clear agar and this poured into a petri dish and the spores allowed to germinate for 24 hours. This method was also unsuccessful as it was very difficult to see the minute

spores except those on the surface. This made it impossible to distinguish them from bits of foreign matter. The difficulties from the sticking together of the spores and the low percentage of germination of isolated spores were encountered also in this method and in every other method used.

Spores were then planted on the surface of plates of clear agar. This was done in two ways, (1) by blowing the dry spores into the petri dish, and (2) by pouring a dilute spore suspension in sterile water over the surface of the agar. Some of the spores remained adhering to the surface. After allowing 24 hours for germination, spores that were single, germinated, and far enough removed from other spores were cut out with a flattened loop and transferred to potato dextrose slants. A great majority of these attempts were also unsuccessful because the delicate sporidia and even promycelium often broke off when the spore was removed and the spore seldom grew after being transferred.

The final method also included the planting of spores on the surface of agar plates by the two methods described. In this case potato dextrose agar was used. Single, germinated, isolated spores were located at the

end of 24 hours through the bottom of the dish but instead of cutting them out at once they were marked by a circle of ink and allowed to grow undisturbed. They were watched daily and whenever a second spore germinated within a marked area or a nearby colony grew into it the mark was removed. After several days of growth, the marked, single-spore colonies were cut out of the agar and transferred to potato dextrose slants. This method was fairly successful and single-spore cultures were finally obtained of all the forms that were desired. It had been planned to try to get single sporidial cultures also, but time did not permit this.

The next problem was to find media on which the oat smuts would grow and show a differentiation of physiologic forms. Potato dextrose, carrot, and oat decoction agars were selected. A preliminary test was conducted with these media unacidified and acidified (about one drop of lactic acid to 15 cc. of medium). As the smuts grew fully as well on the acidified plates as on the checks, all the following experiments were run with acidified agar to lessen the possibility of contamination by bacteria. Nothing further was accomplished in this particular experiment because the plates became badly contaminated through unavoidable circumstances.

From time to time in these preliminary culture studies and in the plantings made in the various attempts to isolate single spores, it was noted that some cultures appeared bacterioid while others of the same physiologic form on the same medium appeared wholly or partly mycelioid. It was suspected that these bacterioid colonies might be contaminated with bacteria. Bits of material from a number of them were examined microscopically and found to contain no bacteria. They had this appearance due to the growth of conidia to the exclusion of mycelium. Why they should produce only conidia when other cultures under the same conditions produced mycelioid growth is not known.

New plantings were made on the three media, all acidified, data recorded, and a photograph taken of the series at the end of twenty-two days (Plates II and III).

Later plantings were made of the five forms, this time in triplicate. Oat meal decoction and prune decoction agars were added to the three previously used. The plantings were not made from the same cultures as before because single spores of L_2 and L_3 had not yet been isolated and consequently mass cultures had been used. It was thought best to use later isolations of the other forms as they were more likely to be from single spores due to improved technique.

The oat meal agar cultures were discarded as there was no apparent difference between the physiologic forms, and the growth was not so abundant as on the other media. The other cultures were grown for a month or more. Since observations showed that variations within a physiologic form were fully as great as differences between forms, their characters have not been recorded.

Plantings again were made in triplicate on acidified potato dextrose, carrot, oat, and prune agars, using the same cultures as in the last experiment. It was hoped to eliminate or find reasons for the inconsistencies between replicate transfers of the same culture. They were planted on the same day and transferred from the same cultures as those on synthetic media referred to later so that the rate of growth under identical conditions could be compared. When the culture plates were a week old, they accidentally became badly contaminated with Penicillium sp. so that all plates had to be discarded. Time did not permit starting new Petri dish cultures; therefore, the question of differences in physiologic forms in culture on vegetable agar media had to remain unanswered.

As previously mentioned, vegetable decoction media cannot be duplicated exactly because of variations in the

plant materials. This necessitates the use of synthetic media for accurate results.

Besides producing good growth of the organism, a synthetic medium must be made of pure chemicals that can be accurately duplicated, must have few constituents, must produce little or no precipitate, and must have a hydrogen-ion concentration after sterilization that is satisfactory for the growth of the organism without the addition of either acid or alkali to adjust the reaction. With these points in mind, Ranker (24) tried many combinations of chemicals in searching for a medium for U. zeae. Many were unsatisfactory because of precipitates or unfavorable hydrogen-ion concentrations. Finally twenty-six were selected for more detailed study. Carrot decoction was used for the control. The cultures were grown for a definite length of time, three weeks in most cases. The centrifuge method was used for measuring the amount of growth. It was found that as much growth occurred in media Nos. 1, 7, 12, 17, and 25 as in carrot decoction, and more occurred in medium No. 7. Finally, Nos. 1, 7, 12, 17, and 25 were selected on which to grow U. avenae and U. levis in the studies discussed herein. They are referred to as R_1, R_7, R_{12}, R_{17}, and R_{25} respectively.

The constituents were as follows:

Culture Medium R_1	
K_2HPO_4	.25 gm.
KCl	.15 gm.
$NaNO_3$	.25 gm.
$MgSO_4.7H_2O$	.2 gm.
$CaSO_4.2H_2O$	.1 gm.
Dextrose	10.0 gm.
Agar	15.0 gm.

Culture Medium R_7	
K_2SO_4	.3 gm.
NH_4NO_3	.1 gm.
$CaCl_2$	.1 gm.
$Mg_3(PO_4)_2.4H_2O$	.1 gm.
Dextrose	10.0 gm.
Agar	15.0 gm.

Culture Medium R_{12}	
KH_2PO_4	.2 gm.
$MgSO_4.7H_2O$	.2 gm.
KNO_3	.2 gm.
NaCl	.2 gm.
$CaSO_4.2H_2O$	.1 gm.
Ferric phosphate soluble scales	.1 gm.
Dextrose	10.0 gm.
Agar	15.0 gm.

Culture Medium R_{17}	
KH_2PO_4	.2 gm.
$MgSO_4.7H_2O$	.2 gm.
KNO_3	.2 gm.
$CaCl_2$	.1 gm.
Na_2SO_4	.1 gm.
Dextrose	10.0 gm.
Agar	15.0 gm.

Culture Medium R_{25}

$MgSO_4.7H_2O$	.2 gm.
KNO_3	.2 gm.
$CaH_4(PO_4)_2.2H_2O$	.2 gm.
$MnSO_4.2H_2O$	.1 gm.
$FeCl_3$	.1 gm.
Dextrose	10.0 gm.
Agar	15.0 gm.

Each chemical was dissolved in a small amount of distilled water and these were then added to the remainder of the water. Although Ranker used them as nutrient solutions only, agar was added to make a solid medium for these experiments.

The only reference found to growing oat smut on synthetic media was by Dickinson (9). The information was incomplete, the formula being given as follows:

KHPO	1 gm.
MgSO	½ gm.
KCl	½ gm.
Agar	15 gm.
Distilled water	1 liter
Maltose	.0156 molecular
Urea	.00195 molecular

That might be interpreted as either of two formulae as follows:

Culture Medium D_1		Culture Medium D_2	
K_2HPO_4	1 gm.	KH_2PO_4	1 gm.
$MgSO_4$	½ gm.	$MgSO_4$	½ gm.
KCl	½ gm.	KCl	½ gm.
Agar	15 gm.	Agar	15 gm.
Distilled water	1 liter	Distilled water	1 liter
Maltose	.0156 molecular	Maltose	.0156 molecular
Urea	.00195 molecular	Urea	.00195 molecular

An agar medium was made up with each form of phosphate, all other ingredients being the same. The .0156 molecular maltose was approximately 5.3 gm. per liter and .00195 molecular urea was approximately .125 gm. per liter.

Plates were poured from each of the synthetic media except R_{25}. The consistency of this was too thin and it could not be used. Preliminary plantings of one form each of U. avenae and U. levis were made on each of these media in order to be certain that oat smut would grow on them.

Satisfactory growth occurred on all the media used. Since it seemed a little better on D_2 than on D_1 and since the two media were very much alike it was decided not to use D_1. Triplicate plantings of single chlamydospore cultures of each of the two forms of U. levis and the three

forms of U. avenae were made on R_1, R_7, R_{12}, R_{17}, and D_2. They were allowed to grow at a temperature of approximately 24° C. for four weeks, when observations were made. These are discussed under experimental results and part of the data is recorded in Table VIII, page 86. They are also illustrated in Plate 4.

EXPERIMENTAL RESULTS

A Study of Spore Markings and their Relation to Panicle Characters

Collections in the Herbarium of the Department of Botany and Plant Pathology. It was natural to expect that the specimens in the mycological herbarium of the Department of Botany and Plant Pathology would be identified and labeled correctly. A study of them would thus show the proper appearance and spore markings of each species. These should be an aid to a study of collections, the species of which had not been determined. However, this was not the case as shown by specimens Nos. 4, 6, 8, 11, 12, 13, and 15 in Table I. In each case they were either labeled incorrectly or contained both species of oat smut.

The error in No. 12 is easily explained by the presence of both species which probably looked very much alike

before the panicles became very ripe and dry. Perhaps the same explanation would hold for No. 4, but there seems to be no way of explaining the error in No. 6. The labeling of collections of U. avenae as U. levis was perhaps due to studying the spores with too low powered a microscope, which did not show the minute echinulations. This error occurred in Nos. 8, 11, 13, and 15.

While this may be too small a number of specimens from which to draw a definite conclusion concerning the relation of spore markings and panicle characters, it would seem that a thin, light-colored membrane over the spore masses is almost always present in U. levis and is a much more reliable distinguishing character than the portion of the glume affected, as depended upon by most of the writers on the subject with the exception of Kellerman and Swingle (18).

This study clearly demonstrated the difficulty encountered, even by experienced mycologists, in distinguishing between the two species of oat smut, and bears out the contention of Melchers and Johnston that the field symptoms of the two species of oat smut are not a definite or reliable character in many cases for their correct determination. It seems very likely from the data secured that the misnamed specimens were labeled on the basis of gross panicle

characters. If the spores were examined by the collectors, microscopes with very poor powers of definition must have been used. If a situation such as this exists in other herbaria, there can be no question of the necessity of further work on the problem of accurate identification of the species of oat smut.

Table I.--Panicle and spore characters of specimens of oat smut in the mycological herbarium of the Department of Botany and Plant Pathology of the Kansas State College of Agriculture and Applied Sciences.

No.	Collection	Species as labeled	Appearance of panicle	Spore wall characters
1	Kellerman and Swingle, Manhattan, Kans., July, 1890.	U. avenae	Glumes shredded and almost completely replaced by spore masses. Many spikelets shed.	Echinulate.
2	E. H. Kern, Mankato, Kans., June 23, 1891.	U. avenae	Glumes and shape of spikelets almost wholly destroyed; less smut toward tip of panicle. All covered with loose, velvety mass of spores.	Echinulate.
3	O. A. Stevens, Northville, S. Dak., July 3, 1929.	U. avenae	Glumes, except tips and narrow margins, replaced by dark brown powdery spore masses.	Distinctly echinulate.
4	F. Bubák, Tabor, Bohemia, Feb. 6, 1906.	U. avenae	Glumes unaffected except for black spore mass at base of each.	Majority smooth. A few echinulate.
5	B. B. Higgins, West Raleigh, N. C., May, 1909.	U. avenae	Glumes affected except tips and margins, brown, somewhat shredded.	Distinctly echinulate.
6	E. B., Seattle, Wash., Aug. 7, 1909.	U. avenae	Glumes affected only at bases. Outer membrane intact on some spikelets; others darker and somewhat powdery.	Smooth.

7	Kellerman and Swingle, Manhattan, Kans., July, 1889.	U. avenae var. levis	Glumes affected only at bases. Light colored membrane covers spore masses.	Smooth.
8	E. Bartholomew, Rooks Co., Kans., Sept. 7, 1894.	U. avenae var. laevis	Base to lower half of glumes affected, somewhat shredded.	Very finely echinulate.
9	C. W. Coman, Americus, Kans., June 23, 1889.	U. avenae var. laevis	Glumes affected except at tips and margin; dark grey-brown from dark spore masses showing through thin outer membrane. At base of panicle spikelets are small, hard, black except tips of glumes.	Smooth.
10	O. A. Garrett, Salt Lake City, Utah, July 15, 1903.	U. levis	Portion of glumes affected varies from base to all except tips, affected most severely at base of panicle. Spikelets are very hard; some are black and sclerotia-like; some grayish and mottled from persistence of light outer membrane.	Smooth.
11	E. T. Bartholomew, Madison, Wis., Oct., 1911.	U. levis	Glumes affected except tips. Shredded, powdery.	Echinulate.
12	T. Lydow. Tommern, Gutsdorf bei Callies, 1897.	U. levis	Most of the panicles: Lower half of glumes affected, dark gray, intact.	Smooth.
			Some panicles: Lower half of glumes shredded, powdery.	Echinulate.

13	W. P. Carr, San Antonio, Texas, April 17, 1910.	U. levis	Glumes affected except tips and margins. Shredded, powdery.	Echinulate.
14	E. T. and E. Bartholomew, Laramie, Wyo., Aug. 11, 1911.	U. levis	Glumes except tips and margins dark silvery gray.	Smooth.
15	C. A. Ludwig, Lafayette, Ind., June 26, 1913.	U. levis	Glumes almost entirely shredded and replaced by dark brown powdery spore masses.	Distinctly echinulate.

Collections from Experiment Stations in Several States. In the results of the studies which are shown in Table II, the following facts are revealed: It is possible to identify the two species correctly in most cases when one is permitted to select typical specimens. Of the 24 collections, No. 13 is the only one that is wholly wrong and in that case the collector expressed his doubt. Numbers 7, 14, 20, and 24 were incorrect in that they contained both species of oat smut. In at least part of those cases, the collector probably made a correct decision, but assumed that only one species was present and for this reason did not take note of every panicle collected.

The writer, basing her decision upon the presence of a light-colored outer membrane, made a wrong decision in the case of only three panicles. These were in collections Nos. 20 and 24. Number 6 could not be identified on the basis of the membrane referred to because there was almost no visible smut. The writer's error in Nos. 20 and 24 indicates that the membrane cannot be relied upon in all cases as a distinguishing character.

As indicated in Table II, several mounts of otherwise smooth spores contained a few echinulate spores. In the case of Nos. 20, 24, and 30 both species of smut were found in the same packet and contamination would be expected. Numbers 2 and 22 might be explained by contamination in the field or in the laboratory. Number 13 indicates that poorly developed or immature spores, as revealed by a misshapen or collapsed condition, may not develop the normal wall markings.

Table II.--Panicle and spore-wall characters of specimens of oat smut in collections from several states in 1930.

No.	Collection	Species indicated by collector	Writer's determination	Appearance of panicle	Spore wall characters
1	B. Koehler, Urbana, Ill.	Loose.	Loose.	Glumes affected except margins. Some shredding of glumes. Covered with powdery masses of spores.	Echinulate.
2	B. Koehler, Urbana, Ill.	Covered.	Covered.	Glumes affected at base and streaks toward tip. Grayish membrane present.	Smooth (two echinulate spores in mount).
3	C. O. Johnston, Dunseith, N. Dak.	Covered.	Covered.	Lower half to all but margins of glumes affected. Grayish membrane present. No shedding of spores or shredding of glumes.	Smooth.
4	C. O. Johnston, Manitou, Man.	Covered.	Covered.	Same as No. 3.	Smooth.
5	M. M. Hoover, Morgantown, W.Va.	Loose.	Loose.	Some glumes almost wholly affected; some at base to lower half. Covered by mass of dark brown powdery spores.	Echinulate.

6	M. M. Hoover, Morgantown, W.Va.	Covered.	Not determined.	Base of glumes affected. Almost no visible smut.	Smooth.
7	C. S. Holton, Owatonna, Minn.	Covered.	Loose.	Glumes affected except margins. Considerable shredding of glumes.	Echinulate.
			One panicle smooth:	Glumes intact. Grayish membrane present.	Smooth.
8	C. S. Holton, Wisc.	Loose.	Loose.	Glumes almost wholly affected. Covered with dark brown powdery mass of spores.	Echinulate.
9	C. S. Holton, Mankato, Minn.	Loose.	Loose.	Same as No. 5.	Echinulate.
10	L. W. Durrell, Fort Collins, Colo.	Not indicated.	Loose.	Mostly base rachises. Lower half of glumes affected; shredded.	Echinulate.
11	L. W. Durrell, Fort Collins, Colo.	Not indicated.	Loose.	Glumes affected except margins. Much shredding of glumes. Covered with powdery masses of spores.	Echinulate.
12	C. B. Cross, Stillwater, Okla.	Probably loose.	Loose.	Glumes affected at base to wholly affected. Much shredding of glumes.	Echinulate.

13	C. B. Cross, Stillwater, Okla.	Possibly covered.	Loose.	Lower half of glumes affected; somewhat shredded. Less smut at tip of panicle.	Mostly echinulate. Some were misshapen or shrunken and some of them were smooth.
14	C. B. Cross, Stillwater, Okla.	Probably loose.	Loose.	(1) Glumes affected at base to all but margins. Considerable shredding of glumes. Powdery spores.	Echinulate.
		Possibly covered.	Loose.	(2) Glumes affected at base to lower half. Slight amount of shredding.	Echinulate.
15	D. C. Smith, Corvallis, Ore.	Loose.	Loose.	Glumes almost wholly affected; covered with dark brown powdery masses of spores. Much shredding.	Echinulate.
16	D. C. Smith, Corvallis, Ore.	Loose.	Loose.	Same as No. 15.	Echinulate.
17	D. C. Smith, Corvallis, Ore.	Covered.	Covered.	Glumes affected at base to lower half; silvery gray. Practically no shedding of spores or shredding.	Smooth.
18	D. C. Smith, Corvallis, Ore.	Covered.	Covered.	Lower half to all but margins of glumes affected; silvery; not shredded. Practically no shedding of spores.	Smooth.

19	Loren Davis, Aberdeen, Idaho.	Loose.	Loose.	Lower half to all but margins of glumes affected. Considerable shredding of glumes. Covered with mass of powdery spores.	Echinulate.
20	Loren Davis, Aberdeen, Idaho.	Covered.	4 panicles covered, remainder loose.	Base to all but margins of glumes affected. Four panicles--grayish membranes present. Four panicles--no shedding of spores or shredding but grayish membrane absent. Three panicles--grayish membrane absent. Some shedding of glumes.	3 smooth. 3 " (1 or 2 echinulate spores in each mount). 5 echinulate.
21	W. E. Brentzell, Barnesville, Minn.	Loose.	Loose.	Glumes affected except margins, shredded, covered with powdery spore masses.	Echinulate.
22	W. E. Brentzell, Barnesville, Minn.	Covered.	Covered.	Lower half to all but margins of glumes affected. Practically no shedding of spores or shredding of glumes. Grayish membrane present.	Smooth (a few echinulate spore in mount).
23	W. H. Leonard, Lincoln, Nebr.	Loose.	Loose.	Glumes affected except margins, shredded, covered with powdery masses of spores.	Echinulate.

24	W. H. Leonard, Lincoln, Nebr.	Covered.	Covered.	Glumes affected at base to all but margins. Basal spikelets reduced, black. Grayish membrane over others.	Smooth (a few echinulate in mount).
			Covered.	Grayish membrane present.	Smooth.
			Loose.	All spikelets reduced. Grayish membrane almost wholly absent.	Smooth.
			Loose.	Grayish membrane absent. Some shredding of glumes.	Echinulate.
25	H. C. Murphy, Ames, Iowa.	Loose.	Loose.	Most of the glumes wholly affected; covered by powdery spore masses.	Echinulate.
26	H. C. Murphy, Ames, Iowa.	Loose.	Loose.	Same as No. 25.	Echinulate.
27	H. C. Murphy, Ames, Iowa.	Loose.	Loose.	Lower half to whole glumes affected, considerable shredding, covered by powdery spore masses.	Echinulate.
28	H. C. Murphy, Ames, Iowa.	Loose.	Loose.	Same as No. 27.	Echinulate.

29	H. C. Murphy, Ames, Iowa.	Covered.	Loose.	Glumes affected at base to lower half. Considerable shedding of spores.	Echinulate.
30	H. C. Murphy, Ames, Iowa.	Covered.	Loose.	Same as No. 29.	Echinulate.
31	H. C. Murphy, Ames, Iowa.	Covered.	Covered.	Base to lower half of glumes affected. Light membrane present.	Smooth (1 echinulate spore in mount).

It was thought that additional interesting information might be gained by inquiring as to the prevalence of the two species in the states where the collections were made. This was done but no definite results were obtained. Several of the investigators overlooked the question or neglected to answer it and two others did not know which was more prevalent, leaving only six definite answers. These are given in Table III. They seem to indicate that no general statement can be made as to the prevalence of one species or the other in any section of the county. It would seem that U. avenae is more prevalent in the south and U. levis in parts of the north. The fact that several of the investigators did not know which was more prevalent in their localities is just another evidence that the species are not easily distinguished and all too little is known of them even by investigators in the field.

Table III.--Statements made by experiment station workers on the probable prevalence of oat smuts in several states.

State	Species more prevalent	Opinion of	Remarks
Illinois	U. levis	B. Koehler	"I believe there is no question ...."
Iowa	U. avenae	H. C. Murphy	Probably 85 per cent.
Minnesota	U. avenae	C. S. Holton	Not answered with absolute certainty.
Nebraska	Did not know	G. L. Peltier	
North Dakota	U. avenae	W. E. Brentzel	"In my opinion..."
Oklahoma	Did not know	F. M. Rolfe	
Oregon	U. levis	D. C. Smith	About 5 to 1. The two species are hard to distinguish.
West Virginia	U. avenae	M. M. Hoover	Probably 75 per cent.

In Table II the results are recorded of fairly successful efforts to assign a number of panicles to the correct species without spore examination. These were undoubtedly selected as typical panicles. Table IV, on the other hand, records efforts to assign a large number of panicles selected at random and containing many intermediate

characters to the correct species by the same method. Selections for study from each group in which they were tentatively placed by the collectors were numbered beginning with 1. Those from the group labeled "loose" were designed by "L," those from the "intermediate" group "I," and those from the "covered" by "C." In contrast to the studies recorded in Table II, where almost every decision was correct, a large number of these panicles were tentatively placed in the wrong species. Every selection from the "covered" group, C No. 1 to C No. 24 inclusive, were found by spore examination to be loose smut despite the fact that the eight panicles, C No. 17 to C No. 24, were not selected at random as all the others had been, but were selected as most nearly resembling the writer's conception of covered smut. Also, all those from the intermediate group, I No. 1 to I No. 15, proved to be loose smut. If they had been placed in this group on the basis of a correct set of distinguishing characters approximately half of them should have been covered smut. Judging from the types of panicles placed in each of the three groups, they were evidently separated on the basis of some such description as this: U. levis - glumes not more than one-half affected, little or no shredding of glumes or shedding of

spores. U. avenae - glumes almost wholly affected, considerably shredded, more or less shedding of powdery spores. While this description corresponds very closely to those given by most of the writers on the subject, it has proved in these studies to be almost a complete failure as a means of separating loose and covered smut. The writer gave her opinion of each panicle, basing her judgment on the belief that a whitish membrane is present in covered smut. There were only four doubtful cases, Nos. I No. 3, I No. 11, I No. 12, and C No. 13, in the group studied.

As in previous studies there were very few panicles containing more than one kind of spores. Panicles L No. 6, C No. 5, C No. 19, and C No. 21 contained some spores on the walls of which echinulations could not be detected but in every case they were shrunken or misshapen, indicating an abnormal development rather than the mixing of the two species.

Table IV.--Panicle and spore-wall characters of collections of oat smut made by L. E. Melchers and C. O. Johnston at the Kansas Agricultural Experiment Station, 1925.

Panicle No.	Collector's determination	Writer's determination	Appearance of panicle	Spore wall markings
L_1	Loose.	Loose.	Glumes affected except narrow margins on a few. Spikelets reduced, powdery.	Echinulate.
L_2	Loose.	Loose.	Glumes affected except narrow margins. Some spikelets shed; those present reduced, powdery.	Echinulate.
L_3	Loose.	Loose.	Glumes affected except narrow margins. Spikelets reduced, powdery.	Echinulate.
L_4	Loose.	Loose.	Same as L_3.	Echinulate.
L_5	Loose.	Loose.	Same as L_3.	Echinulate.
L_6	Loose.	Loose.	Glumes affected except narrow margins. Spikelets much reduced, somewhat hard.	Some misshapen, collapsed. These are smooth or indistinctly echinulate. All others echinulate.
L_7	Loose.	Loose.	Same as L_3.	Echinulate.
L_8	Loose.	Loose.	Same as L_3.	Echinulate.

L_9	Loose.	Loose.	Same as L_1.	Echinulate.
L_{10}	Loose.	Loose.	Same as L_1.	Echinulate.
L_{11}	Loose.	Loose.	Same as L_1.	Echinulate.
L_{12}	Loose.	Loose.	Same as L_3.	Echinulate.
L_{13}	Loose.	Loose.	Glumes affected except margins, Tips, streaks, and wider margins unaffected at tip of panicle. Spikelets at tip of panicle hard, others powdery.	Echinulate
L_{14}	Loose.	Loose.	Same as L_3.	Echinulate.
L_{15}	Loose.	Loose.	Glumes affected except margins. Spikelets reduced, especially at base of panicle; somewhat powdery.	Echinulate.
I_1	Intermediate	Loose.	Glumes from less than one half to all but margins affected. Powdery.	Echinulate.
I_2	Intermediate	Loose.	Same as I_1.	Echinulate.
I_3	Intermediate	Uncertain	Glumes affected except tips and margins. Spikelets much reduced, especially at base of panicle. At tip of panicle black spore masses are partly covered by a membrane; at base of panicle they are powdery.	Echinulate.
I_4	Intermediate	Loose.	Glumes almost wholly affected at base of panicle; affected at base and streaks upward at base of panicle.	Echinulate

I_4 (cont.)			Spikelets much reduced at base of panicle.	
I_5	Intermediate	Loose.	Same as I_4.	Echinulate.
I_6	Intermediate	Loose.	Lower half or more of glumes affected and streaks upward. Most spikelets powdery; some hard.	Echinulate
I_7	Intermediate	Loose.	Most glumes affected except tips and margins; less at tip of panicle. Powdery.	Echinulate
I_8	Intermediate	Loose.	Same as I_4.	Echinulate.
I_9	Intermediate	Loose.	At base of panicle, glumes affected except tips. At tip of panicle, glumes affected only at base. Spikelets reduced, powdery, some absent.	Echinulate.
I_{10}	Intermediate	Loose.	Lower half and streaks upward of most glumes affected. Spikelets at base of panicle powdery, others hard.	Echinulate.
I_{11}	Intermediate	Uncertain	Glumes affected except margins. Spikelets reduced, especially at base of panicle. An outer membrane present in places.	Echinulate.
I_{12}	Intermediate	Uncertain	Glumes affected except margins. Some spikelets shed at base of panicle.	Echinulate.
I_{13}	Intermediate	Loose.	Glumes affected except margins. Spikelets misshapen, somewhat shredded.	Echinulate.

I_{14}	Intermediate	Loose.	Glumes affected except margins. Spikelets reduced, especially at base of panicle.	Echinulate.
I_{15}	Intermediate	Loose.	Glumes at base of panicle wholly affected; others affected except tips and margins. Spikelets reduced at base of panicle; powdery.	Echinulate.
C_1	Covered.	Loose.	Base of glumes affected, powdery. Some spikelets shed.	Echinulate.
C_2	Covered.	Loose.	Base of glumes and streaks upward affected. Spikelets reduced at base; some powdery, majority hard.	Echinulate.
C_3	Covered.	Loose.	Glumes mostly affected at base or lower half. Some at base of panicle affected except margins. Some are powdery.	Echinulate.
C_4	Covered.	Loose.	Base to lower half of glumes affected.	Echinulate.
C_5	Covered.	Loose.	Glumes affected at base and streaks upward. Spikelets much reduced at base of panicle; somewhat powdery.	Many shrunken or misshapen. Some of them were smooth, others echinulate.
C_6	Covered.	Loose.	Glumes affected at base to almost wholly affected. Spikelets reduced at base of panicle.	Echinulate.

C_7	Covered.	Loose.	Glumes affected at base and streaks upward.	Echinulate.
C_8	Covered.	Loose.	Glumes affected at base to almost wholly affected. Spikelets reduced at base of panicle, somewhat powdery.	Echinulate.
C_9	Covered.	Loose.	Base to lower half of glumes affected. Powdery in places.	Echinulate.
C_{10}	Covered.	Loose.	Glumes affected at base and streaks upward or all but margins affected. Most spikelets hard, a few absent.	Echinulate.
C_{11}	Covered.	Loose.	Glumes affected at base. Spikelets reduced at base of panicle.	Echinulate.
C_{12}	Covered.	Loose.	Glumes affected at base to almost wholly affected. Spikelets reduced at base of panicle.	Echinulate.
C_{13}	Covered.	Uncertain	Glumes affected at base to almost wholly affected. Spikelets reduced at base of panicle. A light outer membrane present in places.	Echinulate.
C_{14}	Covered.	Loose.	Glumes affected at base and streaks upward. Spikelets reduced at base of panicle.	Echinulate.
C_{15}	Covered.	Loose.	Same as C_{11}.	Echinulate.
C_{16}	Covered.	Loose.	Most glumes affected except margin, shredded.	Echinulate.

C_{17}	Covered.	Loose.	Glumes affected at base to all but margins, not shredded. No shedding of spores.	Echinulate.
C_{18}	Covered.	Loose.	Base of glumes very black, not shredded. No shedding of spores.	Echinulate.
C_{19}	Covered.	Loose.	Most glumes affected at base and streaks upward, not shredded. Spikelets reduced at base of panicle. No shedding of spores.	Some are shrunken, misshapen. These are not echinulate. Others echinulate.
C_{20}	Covered.	Loose.	Most glumes affected at base or lower half and streaks upward, not shredded. Spikelets reduced at base of panicle. No shedding of spores.	Echinulate.
C_{21}	Covered.	Loose.	Glumes affected at base to all but wide margin, not shredded. No shedding of spores.	Some are misshapen. These are not all echinulate. Others echinulate.
C_{22}	Covered.	Loose.	Glumes affected at base and streaks upward, not shredded. No shedding of spores.	Echinulate.
C_{23}	Covered.	Loose.	Base of glumes affected for most part, glumes at base of panicle almost wholly affected; not shredded. No shedding of spores.	Echinulate.
C_{24}	Covered.	Loose.	Glumes affected at base to wholly unaffected, not shredded. No shedding of spores.	Echinulate.

Collections from the Oat Smut Nursery of the Kansas Agricultural Experiment Station. The data recorded in Tables V and VI were obtained from a study of a great many oat panicles from collections of covered, as well as loose, smut. In every study of covered smut, the panicle characters verified the decision based on the earlier studies of a relatively small number of specimens; namely, that a whitish membrane persists over the spore masses in U. levis. The fact brought out in Table IV, that panicle characters such as portion of glumes affected, amount of shredding of glumes, and amount of shedding of spores cannot be used as a means of distinguishing with certainty between the two species, is brought out much more forcibly here. In very many cases the same species on the same oat variety produced widely differing symptoms on different panicles. This is well illustrated by rows 15, 24, 46, 78, 87, 90, 165 and a number of others in Table V. In every case microscopic spore examination revealed that there was not more than one species present.

This finding of several types of panicles in the same collection was just as evident in the 1930 crop as shown in Table VI. Collection 6 illustrates this particularly well. Even though there were an average of only 25 per cent of

the glumes present and a marked shredding of glumes and shedding of spores, still there were from 25 to 50 per cent partially smutted panicles. Many of such panicles had only a small percentage of glumes affected, little shredding of glumes, and little or no shedding of spores. The facts are more striking than the figures in these collections of loose smut. Most of the specimens had glumes 0 to 10 per cent present but some, especially the partially smutted panicles, had glumes 50 to 75 per cent present, bringing the average to about 25 per cent. An explanation for some of the partially smutted heads is probably immaturity since most of them were green. Many of the partially smutted panicles have typical loose smut in a few basal spikelets, with almost complete destruction of glumes and with shedding of spores. The variation in panicle characters within a collection is illustrated by panicles 1 to 5, Plate I. These were all taken from collection 2, row 10 on the same variety, Kanota. Panicle 2 is an aberrant form of Kanota as indicated by the two awns. With such variations in almost any oat collection it is difficult to see how anyone can hope to distinguish the particular species by the amount of visible smut or the portion of the glumes affected.

Panicles 6 and 7 are typical specimens of covered smut taken from collection 1, row 3 and collection 3, row 21, respectively. It can be seen that a large portion of the glumes are affected but the spore masses are held intact and given a grayish color by an outer layer of glume tissue which remains as a thin whitish membrane. The two panicles of loose smut in collection 1 and the one in collection 3 determined by spore examination as well as appearance of panicle, were undoubtedly produced from contamination in the inoculum used in smutting the seed before sowing. Panicle 1, row 37 in Table V can be accounted for in the same manner.

An effort was made to detect differences between collections that infection studies had proved to be different physiologic forms but the only differences noted were slight and inconsistent and probably not significant. The same thing was true of different oat varieties infected by a single collection of smut. There seemed to be a little less shedding of spores in Richland and a little more in Kanota but these differences were not marked and not consistent throughout the different smut collections. Thus it can be said here, too, that there were only slight and probably insignificant differences.

It was not considered necessary to study the spores in the 1930 crop except in a few special cases since they had been carefully examined the preceding year. The 1929 data presented in Table V verify conclusions already reached with smaller numbers. Smooth and echinulate spores seldom were found in the same panicle. Panicle 1 in row 136, which is an exception, can be explained as all those in other studies were, on the basis of poor development. The spikelet had all been shed, leaving almost nothing but bare rachis; consequently, most of the normal, well developed spores were gone.

The spore studies in collections 1 and 3, which proved to be covered smut, gave some difficulty at first. In spite of the fact that all observations were made with an oil immersion lens, it was difficult to determine whether they were smooth or faintly echinulate. Most of them had very granular contents giving a dotted effect and it was not until two or more observations had been made with two different microscopes that it was decided for certain that these dots were in the interior of the spore and not on the surface of the wall. Indications are, then, that the species can be determined accurately by spore examination only if studied carefully with high magnification.

Table V.--Panicle and spore-wall characters of specimens of oat smut from the Kansas Agricultural Experiment Station, 1929.

Collection	Row	Appearance of panicle	Spore-wall characters
1	1	Spore masses only at base of spikelets and streaks toward tip, covered by whitish to brownish membrane.	Smooth.
	2	Same as row 1.	Smooth.
	3	Glumes affected except at tips and margins. Whitish membrane present.	Smooth.
	4	At base of panicle, glumes affected except tips. At tip of panicle, glumes affected at base.	Smooth.
	5	No smut.	
	6	Same as row 4.	
	7	At base of panicle, glumes affected except tips. At tip of panicle, glumes affected at base. Spikelets at base of panicle much reduced.	Smooth.
	8	Same as row 4.	Smooth.
	9	No smut.	
	10	Base and streaks upward to lower half of glumes affected. Some spikelets shed.	Smooth.
	11	Base and streaks upward to lower half of glumes affected.	Smooth.

	12	No smut.	
	13	(1) Glumes affected except tips and margins. Spikelets hard.	Smooth.
		(2) Glumes black and somewhat shredded at base of panicle. Elsewhere covered by whitish membrane.	Smooth.
2	14	(1) Glumes mostly affected. Spikelets hard.	Echinulate.
		(2) At base of panicle, glumes affected except tips; shredded. Tip of panicle healthy.	Echinulate.
	15	(1) At base of panicle, glumes affected at base to all except tips. Tip of panicle healthy.	Echinulate.
		(2) All spikelets shed except for a few shreds.	Echinulate.
	16	Glumes affected except tips; shredded. Many spikelets shed.	Echinulate.
	17	Glumes affected except tips at tip of panicle; covered by powdery spore masses.	Echinulate.
	18	No smut.	
	19	(1) Spikelets shed except at tip of panicle. Glumes wholly affected.	Echinulate.
		(2) At base of panicle, spikelets much reduced. Glumes affected except tips. Tip of panicle healthy.	Echinulate.
	20	Same as row 17.	Echinulate.
	21	Glumes affected except tips at tip of panicle; covered by powdery spore masses. Some spikelets reduced; some shed.	Echinulate

	22	No smut.	
	23	(1) At base of panicle glumes affected except tips; powdery. Rest of panicle healthy.	Echinulate.
		(2) Glumes affected except tips at tip of panicle.	Echinulate.
	24	(1) Glumes wholly affected. Many spikelets shed.	Echinulate.
		(2) Base of glumes affected at base of panicle. Otherwise healthy.	Echinulate.
	25		
	26	(1) Glumes affected except a few tips. Some spikelets shed.	Echinulate.
		(2) At base of panicle, glumes wholly affected, spikelets reduced. Gradually less smut toward tip of panicle. Tip healthy.	Echinulate.
3	27	Glumes at tip of panicle affected at base; others affected except tips and margins. Spikelets hard. Whitish membrane present.	Smooth.
	28	Same as row 27.	Smooth.
	29	Glumes affected except tips and margins. Whitish membrane present.	Smooth.
	30	Glumes affected at base and streak upward. Whitish membrane.	Smooth.
	31	No smut.	
	32	Same as row 30.	Smooth.

	33	(1) Glumes entire. Spikelets much shrunken, unfilled.	Smooth. (2 echinulate in mount.)
		(2) At base of panicle glumes affected except tips and margins. Less smut toward tip of panicle. Tip healthy. Whitish membrane present.	Smooth.
	34	Same as 33 (2).	Smooth.
	35	No smut.	
	36	Same as 33 (2).	Smooth.
	37	(1) All spikelets shed except shreds at tip of panicle.	Echinulate.
		(2) Most glumes affected except tips and margins. Less smut at tip of panicle. Whitish membrane present.	Smooth.
7	75	Glumes affected except tips and margins; shredded and powdery.	Echinulate.
	76	Glumes affected except tips and margins; shredded and powdery. Spikelets at base of panicle reduced.	Echinulate. Echinulations absent or indistinct on misshapen spores.
	77	Glumes affected except tips and margins; shredded and powdery. Spikelets at base of panicle reduced. Less smut at tip of panicle.	Echinulate.
	78	(1) Base of glumes at base of panicle affected.	Echinulate.
		(2) Same as row 75.	Echinulate.

	79	No. smut.	
	80	Same as row 76.	Echinulate.
	81	Tip of panicle healthy. Near tip only base of glumes affected. On remainder of panicle glumes affected except tips and margins; shredded and powdery spikelets at base of panicle reduced.	Echinulate.
	82	Same as row 78 (1).	Echinulate.
	83	No smut.	
	84	Glumes affected except tips at tip of panicle.	Echinulate.
	85	Same as row 75.	Echinulate.
14	159	Glumes completely affected except a few tips; shredded. Many spikelets shed.	Echinulate.
	160	Almost all spikelets shed.	Echinulate.
	161	Spikelets shed from base of panicle. Glumes entirely present. Black at extreme base of a few.	Echinulate.
	162	No smut.	
	163	No smut.	
	164	Same as row 159.	Echinulate.
	165	Spikelets from lower half of panicle shed. All those present are healthy.	No spores.

	:166	:Same as row 159.	:Echinulate.
	:167	:No smut.	:
	:168	:(1) Same as row 165.	:Echinulate.
	:	:(2) Tip of panicle healthy. In remainder of panicle	:Echinulate.
	:	: glumes vary from completely affected to only	:
	:	: at base and streaks upward.	:
	:169	:(1) Practically all spikelets shed. Those present	:Echinulate.
	:	: much reduced and glumes completely affected.	:
	:	:(2) Upper part of panicle healthy. At base of	:Echinulate.
	:	: panicle, glumes affected at base and streaks	:
	:	: upward or lower half affected. Not powdery.	:
4	:39-50	:Glumes almost wholly affected. Some panicle tips	:Echinulate.
	:inoculum	:healthy. See row 46.	:
	: 46	:(1) Glumes almost wholly affected, shredded.	:Echinulate.
	:	:(2) Glumes at base of panicle black at base and	:Echinulate.
	:	: streaks upward. Remainder of panicle healthy.	:
5	:51-62	:Glumes from one-half to wholly affected. Some	:Echinulate.
	:inoculum	:specimens firm, not powdery, might be suspected	:
	:	:of being covered smut. One panicle tip healthy.	:
6	:63-74	:In most cases, glumes wholly or almost wholly	:Echinulate.
	:inoculum	:affected. A few panicle tips healthy.	:
8	:87-98	:Glumes mostly affected. Tips of some panicles	:Echinulate.
	:inoculum	:healthy. See rows 87, 90.	:
	: 87	:(1) At base of panicle glumes black at base and	:Echinulate.
	:	: streaks upward. Remainder of panicle healthy.	:

		:(2) Glumes affected except tips and edges; powdery and shredded.	:Echinulate.
	90	:Base of glumes at base of panicle affected. :Remainder of panicle healthy.	:Echinulate.
9	:inoculum:	:99-110:Glumes almost completely destroyed. See rows 104, 105.	:Echinulate.
	104	:Lower half of glumes affected. Upper half of :panicle healthy.	:Echinulate.
	105	:At base of panicle glumes blackened a little at :base and black showing through above. Remainder of :panicle healthy.	:Echinulate.
10	:inoculum:	:111-122:Glumes almost wholly affected. Powdery. Some :panicle tips healthy. See row 113.	:Echinulate.
	113	:Base of glumes at base of panicle black. Remainder :of panicle healthy.	:Echinulate.
11	:inoculum:	:123-134:Glumes almost wholly affected. Many spikelets :shed from some panicles. One panicle tip :healthy.	:Echinulate.
12	:in-ocu-lum	:135-146:Glumes almost wholly affected. Some panicle tips :healthy. Many spikelets shed. See rows 136, 138, :140.	:Echinulate.
	136	:(1)Spikelets shed. Remains hard.	:Smooth--faintly dotted-echinulate.
		:(2)Glumes affected except tips and margins at base :of panicle. Remainder of panicle healthy.	:Echinulate.

	:138	:(1) At base of panicle glumes almost wholly affected, very hard. Spikelets reduced.	:Echinulate.
		:(2) Glumes wholly affected, shredded. Many spikelets shed.	:Echinulate.
	:140	:(1) All spikelets shed.	
		:(2) Only base of glumes at base of panicle affected.	:Echinulate.
13	:147-158 :inoculum:	:Glumes mostly affected. Many spikelets shed. Tips of some panicles healthy. See rows 149, 153, 154, 157.	:Echinulate.
	:149	:Basal spikelets shed. At center of panicle glumes slightly blackened at base. Panicle tip healthy.	:Echinulate.
	:153	:Only base of glumesblack. Some only slightly so.	:Echinulate.
	:154	:(1) Glumes affected except tips and margins; shredded.	:Echinulate.
		:(2) At base of panicle, glumes affected at base to lower half. Remainder of panicle healthy.	:Echinulate.
	:157	:At base of panicle glumes affected at base to lower half. Remainder of panicle healthy. Many spikelets shed.	:Echinulate.
15	:171- :inoculum:	:Glumes vary from black at base to wholly affected. Many spikelets shed. Some panicle tips healthy.	:Echinulate.
Paola, Kans., 1929:		Glumes lower half to wholly affected; shredded.	:Echinulate.

Kinsley, Kans., 1929:	Lower half to all but margins of glumes affected; slightly shredded. Some persistence of outer membrane in places.	Echinulate.

Table VI.--Panicle characters of specimens of oat smut from the Kansas Agricultural Experiment Station, 1930.

Collection No.	Oat variety	Row No.	Per cent glumes present	Shredding of glumes	Shedding of spores	Color of glumes	Per cent partially smutted panicles	Remarks.
1	Kanota.	1	95	None.	Practically none.	Silvery.	0	Two panicles loose smut with shredded glumes. All others typical covered smut.
	Red Texas.	2						
	Richland.	3	95	Very slight	Practically none.	Silvery.	1	Typical covered smut.
	Markton.	4	95	Very slight	Practically none.	Silvery.	0	Typical covered smut.
	Fulghum.	5	95	Very slight	Practically none.	Silvery.	0	Typical covered smut.
	Red Rustproof.	6	95	Very slight	Practically none.	Silvery.	0	Typical covered smut.
	Frazier.	7	95	Very slight	Practically none.	Silvery.	0	Typical covered smut.
	Navarro.	8						
	Nortex.	9						
2	Kanota.	10	25	Marked.	Very marked.	Papery white.	20	Typical loose smut. (Partially smutted panicles green, smutted at base with typical loose smut.)
	Red Texas.	11						
	Richland.	12						
	Markton.	13						
	Fulghum.	14	25	Marked.	Marked.	Papery white.	15	Typical loose smut.
	Red Rustproof	15	15	Marked.	Marked.	Papery white.	10	Typical loose smut. Shedding of spikelets.
	Frazier.	16	25	Marked.	Very marked.	Papery white.	20	Typical loose smut.
	Navarro.	17						
	Nortex.	18						

3	Kanota.	19						
	Red Texas.	20						
	Richland.	21	95	Very slight	Very slight	Silvery.	Trace	Typical covered smut.
	Markton.	22	95	Very slight	Very slight	Silvery.	33	Typical covered smut.
	Fulghum.	23						
	Red Rustproof	24						
	Frazier.	25	95	Very slight	Very slight	Silvery.	33	Typical covered smut. (1 panicle of loose smut present.)
	Navarro.	26						
	Nortex.	27						
4	Kanota.	28	25	Marked.	Marked.	Papery white	5	Typical loose smut. Shedding of spikelets.
	Red Texas.	29						
	Richland.	30	25	Marked.	Moderate.	Papery white	15	Typical loose smut. Partially smutted heads are green with typical loose smut at base.
	Markton.	31						
	Fulghum.	32	25	Marked.	Marked.	Papery white	10	Typical loose smut. Shedding of spikelets.
	Red Rustproof	33	25	Marked.	Marked.	Papery white	0	Typical loose smut. Shedding of spikelets.
	Frazier.	34						
	Navarro.	35						
	Nortex.	36						
5	Kanota.	37	25	Marked.	Very marked	Papery white	0	Typical loose smut.
	Red Texas.	38						
	Richland.	39	25	Marked.	Moderate.	Papery white	5	Typical loose smut.
	Markton.	40						
	Fulghum.	41						
	Red Rustproof	42	15	Marked.	Very marked	Papery white	0	Typical loose smut. Shedding of spikelets.

	Frazier.	43						
	Navarro.	44						
	Nortex.	45						
6	Kanota.	46	25	Marked.	Very marked	Papery white	25	Typical loose smut.
	Red Texas.	47						
	Richland.	48	25	Marked.	Moderate.	Papery white	25	Typical loose smut.
	Markton.	49						
	Fulghum.	50	25	Marked.	Marked.	Papery white	25	Typical loose smut.
	Red Rustproof	51						
	Frazier.	52	25	Marked.	Marked.	Papery white	50	Typical loose smut.
	Navarro.	53						
	Nortex.	54						
7	Kanota.	55	50	Marked.	Marked.	Papery white	10	Typical loose smut. Shedding of spikelets.
	Red Texas.	56						
	Richland.	57	25	Marked.	Marked.	Papery white	10	Typical loose smut.
	Markton.	58	25	Marked.	Marked.	Papery white	10	Typical loose smut.
	Fulghum.	59	25	Marked.	Marked.	Papery white	10	Typical loose smut.
	Red Rustproof	60	25	Marked.	Marked.	Papery white	0	Typical loose smut.
	Frazier.	61	25	Marked.	Marked.	Papery white	25	Typical loose smut.
	Navarro.	62						
	Nortex.	63						
8	Kanota.	64	25	Marked.	Very marked	Papery white	20	Typical loose smut.
	Red Texas.	65	25	Marked.	Marked.	Papery white	2 pan. 100	Typical loose smut.

:Richland.	:66	15	:Marked.	:Marked.	:Papery white	1 pan. 0	:Typical loose smut.
:Markton.	:67						
:Fulghum.	:68	25	:Marked.	:Marked.	:Papery white	25	:Typical loose smut.
:Red Rustproof	:69	25	:Marked.	:Marked.	:Papery white	0	:Typical loose smut. Shedding of spikelets.
:Frazier.	:70	50	:Marked.	:Moderate.	:Papery white	25	:Typical loose smut.
:Navarro.	:71						
:Nortex.	:72						
:Kanota.	:73	25	:Marked.	:Marked.	:Papery white	25	:Typical loose smut.
:Red Texas.	:74						
:Richland.	:75	25	:Marked.	:Marked.	:Papery white	15	:Typical loose smut.
:Markton.	:76						
:Fulghum.	:77	25	:Marked.	:Marked.	:Papery white	35	:Typical loose smut.
:Red Rustproof	:78	15	:Marked.	:Marked.	:Papery white	0	:Typical loose smut.
:Frazier.	:79	25	:Marked.	:Marked.	:Papery white	15	:Typical loose smut.
:Navarro.	:80						
:Nortex.	:81						
:Kanota.	:82	35	:Marked.	:Marked.	:Papery white	15	:Typical loose smut.
:Red Texas.	:83						
:Richland.	:84	25	:Marked.	:Marked.	:Papery white	10	:Typical loose smut.
:Markton.	:85						
:Fulghum.	:86	50	:Marked.	:Marked.	:Papery white	5	:Typical loose smut.
:Red Rustproof	:87	15	:Marked.	:Marked.	:Papery white	0	:Typical loose smut.

	;Frazier.	;88	25	;Marked.	;Marked.	;Papery white;	10	; Typical loose smut.
	;Navarro.	;89						
	;Nortex.	;90						
11	;Kanota.	;91	50	;Marked.	;Very marked;	Papery white;	25	; Typical loose smut.
	;Red Texas.	;92						
	;Richland.	;93	10	;Marked.	;Marked.	;Papery white;	0	; Typical loose smut.
	;Markton.	;94						
	;Fulghum.	;95	25	;Marked.	;Marked.	;Papery white;	20	; Typical loose smut. Shedding of spikelets.
	;Red Rustproof	;96	25	;Marked.	;Moderate.	;Papery white;	20	; Typical loose smut. Shedding of spikelets.
	;Frazier.	;97	25	;Marked.	;Marked.	;Papery white;	50	; Typical loose smut. Shedding of spikelets.
	;Navarro.	;98						
	;Nortex.	;99						
12	;Kanota.	;100;	25	;Marked.	;Marked.	;Papery white;	25	; Typical loose smut.
	;Red Texas.	;101;						
	;Richland.	;102;						
	;Markton.	;103;						
	;Fulghum.	;104;	25	;Marked.	;Marked.	;Papery white;	25	; Typical loose smut. Shedding of spikelets.
	;Red Rustproof	;105;	15	;Marked.	;Moderate.	;Papery white;	0	; Typical loose smut. Shedding of spikelets.
	;Frazier.	;106;	25	;Marked.	;Marked.	;Papery white;	35	; Typical loose smut. Shedding of spikelets.
	;Navarro.	;107;						
	;Nortex.	;108;						
13	;Kanota.	;109;	25	;Marked.	;Marked.	;Papery white;	20	; Typical loose smut.
	;Red Texas.	;110;						

	:Richland.	:111:	25	:Marked.	:Moderate.	:Papery white: 15	: Typical loose smut.
	:Markton.	:112:		:	:	: :	:
	:Fulghum.	:113:	25	:Marked.	:Marked.	:Papery white: 20	: Typical loose smut.
	:Red Rustproof	:114:	15	:Marked.	:Marked.	:Papery white: 30	: Typical loose smut.
	:Frazier.	:115:	50	:Marked.	:Marked.	:Papery white: 50	: Typical loose smut. Mostly green.
	:Navarro.	:116:		:	:	: :	:
	:Nortex.	:117:		:	:	: :	:
14	:Kanota.	:118:	25	:Marked.	:Marked.	:Papery white: 20	: Typical loose smut.
	:Red Texas.	:119:	25	:Marked.	:Marked.	:Papery white: 50	: Typical loose smut.
	:Richland.	:120:		:	:	: :	:
	:Markton.	:121:		:	:	: :	:
	:Fulghum.	:122:	25	:Marked.	:Marked.	:Papery white: 25	: Typical loose smut.
	:Red Rustproof	:123:	50	:Marked.	:Marked.	:Papery white: 30	: Typical loose smut.
	:Frazier.	:124:	25	:Marked.	:Marked.	:Papery white: 25	: Typical loose smut.
	:Navarro.	:125:		:	:	: :	:
	:Nortex.	:126:		:	:	: :	:

Cultural Studies

Several investigators have reported on characteristic and consistent differences between physiologic forms of various smuts grown in culture on liquid or solid media. Christensen and Stakman (4) noted them in U. zeae, Rodenhiser (29) found them in U. tritici, U. nuda, U. hordei, U. levis and U. avenae, and Ficke and Johnston (11) recorded their occurrence in Sphacelotheca sorghi. Kienholz and Heald (20) grew Tilletia tritici and T. levis on several media but many of the differences noted were not consistent.

An effort was made to study the cultural characteristics of U. avenae and U. levis in these experiments with only limited success except in a few cases. Vegetable media were found to be very satisfactory for growing cultures of oat smut and gave striking differences between physiologic forms in a single set of cultures. Owing to a very large amount of contamination in several sets of cultures, and wide variations within physiologic forms in another set, it was impossible to repeat the results. The only cultures on plant decoction media that were carried to completion and described were those planted Feb. 12, 1931,

described March 6, and photographed March 7. The results are given in Table VII and in Plates II and III. Colony measurements were not recorded since there was little variation in size between physiologic forms on the same medium. As shown in these plates, the cultural differences are sufficiently distinct so that one could distinguish all the physiologic forms by that method. Very little significance should be attached to this, however, since triplicate plantings made shortly afterward and grown for about a month reveal differences in size, surface and consistency within physiologic forms that are equal to those between forms. The discovery made by Dickinson (9) that + and — sporidia are produced which may carry contrasting characters may account at least in part for this situation. There would be the possibility of as many as four contrasting single sporidial cultures from one chlamydospore and still others from their fusion.

Table VII.--Cultural studies of U. avenae and U. levis on potato dextrose, carrot, and oat agar media. Planted Feb. 12, described March 6, 1931.

Physiologic form	Color	Elevation and surface	Consistency	Margin
		On Potato Dextrose Agar		
C_1	Whitish.	Raised; smooth.	Bacterioid.	Entire; mycelioid in places.
C_2	Cream with white felt at margins.	Slightly raised. Radial ridges; concentric circles.	Waxy.	Mycelioid.
L_1	Cream.	Raised, rough at center, otherwise almost flat. Fine radial, counter-clockwise ridges near margin.	Center--bacterioid. Near margin--mycelioid.	Mycelioid.
L_2	Cream with white at margin.	Raised, rough at center; otherwise almost flat. Slightly counter-clockwise radial ridges except near margin.	Center--waxy. Near margin--mycelioid.	Mycelioid; irregular.
L_3	Cream with white at margin.	Raised, rough at center. Slightly counter-clockwise radial ridges for entire radius.	Waxy. Mycelioid near margin.	Mycelioid.

On Carrot Agar

C_1	Cream, translucent.	Flat; concentric circles.	Waxy at center. Otherwise mycelioid.	Mycelioid.
C_2	Cream.	Raised, rough at center; otherwise flat. Concentric circles.	Mycelioid.	Mycelioid.
L_1	Cream, translucent.	Raised, rough at center. Very flat near margin. Concentric circles.	Bacterioid center. Mycelioid toward margin.	Entire, mycelioid.
L_2	Whitish to cream.	Slightly raised; rough.	Bacterioid.	Entire.
L_3	Cream.	Raised at center; otherwise very flat.	Waxy.	Entire, mycelioid.

On Oat Agar

C_1	Cream.	Flat except at center.	Mycelioid, felty.	Mycelioid with small lobes.
C_2	White.	Flat except at center.	Felty.	Mycelioid.
L_1	Cream.	Almost flat except at center.	Bacterioid.	Irregular, lobed. Mycelioid in places.

L_2	Cream with white felt in patches.	Almost flat except at center. Radial lines slightly counter-clockwise.	Mycelioid.	Mycelioid.
L_3	White.	Slightly raised.	Felty.	Irregular, dotted, lobed.

The cultures grown on synthetic media produced much more satisfactory results. When they were examined 28 days after planting it was found that on media R_1 and R_{17}, the characters of the different physiologic forms were almost identical, variations between them being no greater than those occurring within a single form. Since they could not be distinguished from one another on these media the cultures were discarded. All were cream colored, very effuse, and spreading.

Growth on Dickinson's medium was better in that it was not so flat. The extent of growth was no greater, however. This medium was also useless for distinguishing physiologic forms, with one exception. In the L_3 cultures, the colonies were smooth and waxy at the center, around which there was a belt of short radial ridges, which in turn was surrounded by a flat mycelial growth at the margin. This set of characters was perfectly constant for the triplicate L_3 cultures and was not found in any other physiologic form. Since no other forms could be distinguished on this medium, these cultures were also discarded.

Physiologic forms could be differentiated on media R_7 and R_{12}, and they were therefore described in Table VIII, using Ridgway's Color Standards and Color Nomencla-

ture (28) for the naming of the colors. Photographs were secured of typical cultures of the 5 physiologic forms on the same two media, showing characteristic differences (Plate IV). Forms C_2, L_1, and L_3 on media R_7 and R_{12} were perfectly constant for the triplicate cultures. Culture C_1 on R_7 as illustrated, was the only one of that appearance. A second resembled C_2 and the third was discarded because of contamination. One of the three cultures of C_1 on R_{12} also resembled C_2. Owing to these varieties in C_1, and the similarity between C_1 and C_2, it is not known whether C_1 and C_2 are separate physiologic forms or not as previously stated. This question was not solved by the infection studies in the field, as it is not known whether the slight differences found in varietal resistance are significant, but it is believed that they are not.

Sectoring has been observed in fungous cultures by many investigators. It usually has been associated with the instability caused by frequent mutation, or segregation. Sectoring is common in cultures of U. zeae, and Rodenhiser (29) noted its occurrence in U. avenae. In the experiments herein discussed, sectoring occurred with regularity only in loose smut culture L_1 on R_{12}. This can probably be explained also on the basis of segregation of characters in

the sporidia, but why it should occur freely in one form and seldom if ever in another is not known. The same phenomenon has been noted, however, by Ficke and Johnston (11) in S. sorghi and by Rodenhiser (29) in U. avenae.

Growth was better on R_7 than on R_{12}, and the differences between physiologic forms were more readily discernible, especially the colors. It would seem from these limited studies that R_7 is a satisfactory medium for growing oat smuts.

Table VIII.--Cultural studies of U. avenae and U. levis on Ranker's Media No. 7 and No. 12. Planted March 25, Described April 21, 1931.
(Triplicate cultures of a single form are similar unless otherwise noted.)

Physiologic form	Color	Elevation and surface	Consistency	Margin
		On Ranker's No. 7		
C_1	(1) Same as C_2.			
	(2) Cream with whitish margin.	Raised; radial ridges.	Bacterioid. Margin mycelioid.	Mycelioid. Fine lines running counterclockwise.
	(3) Discarded because of contamination.			
C_2	Pale buffy brown interspersed with olive brown; margin whitish.	Raised and wrinkled at center for about one-half the diameter.	Waxy center; mycelioid toward margin; bits of white felt in places.	Partly mycelioid; partly "beaded-ciliate," the cilia beaded apparently from the throwing of sporidia.
L_1	Whitish margin; center maize yellow interspersed with orange citrine.	Raised and wrinkled at center for about one-third the diameter; remainder flat.	Waxy center; remainder mycelioid.	Entire; mycelioid.

L_2	Old gold-orange citrine at center; whitish margin.	Raised and wrinkled at center for about one-half the diameter; remainder flat.	Waxy center; remainder mycelioid.	Mycelioid.
L_3	Whitish margin; cream-buff to Isabella color at center.	Raised and wrinkled at center; conspicuous radial ridges.	Waxy center; mycelioid toward margin.	Mycelioid. Fine lines running slightly counter-clockwise.

On Ranker's No. 12

C_1	(1, 2) Buffy brown center through cartridge buff to almost colorless at margin. (3) Same as C_2.	Small area in center much raised and irregular. The remainder flat.	Waxy center; remainder mycelioid. Spots and lines of white felt in places.	Partly mycelioid but mostly "beaded ciliate" --the cilia beaded apparently from the throwing of sporidia.
C_2	Buffy brown center to cream margin. Transparent ring near margin.	Same as C_1.	Waxy center; remainder mycelioid. A few bits of white felt.	"Beaded-ciliate" as in C_1.
L_1	Cream with a little buffy brown in center.	Same as C_1.	Waxy center; remainder mycelioid.	"Beaded-ciliate" as in C_1. Two of the three plantings have more rapidly growing sectors with entire, mycelioid margin.

L_2	Olive brown partly overlaid with white at center to olive buff at margin.	Same as C_1.	Center partly wet, bacterioid; partly overlaid with white felt. Remainder mycelioid.	Mycelioid.
L_3	Cream.	Same as C_1.	Waxy center; remainder mycelioid.	Mycelioid; indistinct.

DISCUSSION

It was found that errors in identifying U. avenae (Pers.) Jens. and U. levis (K. & Sw.) Magn. were frequent in the early collections in the herbarium of the Department of Botany and Plant Pathology of the Kansas State College of Agriculture and Applied Science. This shows that even experienced mycologists were not free from error in separating the species of oat smut. Since this is true for the collections made by such authorities as Kellerman and Swingle and others on smuts, and especially oat smuts, it seems reasonable to believe there are errors in identifying these species in all the mycological herbaria of the United States and foreign countries. This was probably partly due to the fact that high magnification is necessary in order to see the echinulations on the spore walls and partly to identification without spore examination. The several studies of spore markings and their relation to smutted panicle characters indicate that one cannot distinguish the two species with certainty except by spore examination. The portion of the glumes affected, the amount of visible smut, the amount of shredding of the glumes, and the amount of shedding of the spores are not

reliable characters for distinguishing the two species. An outer layer of glume tissue remaining as a whitish layer over the dark spore masses is usually reliable as a distinguishing character for U. levis.

These conclusions are in agreement with the original description of U. levis by Kellerman and Swingle (18). They indicate, however, that the descriptions and distinguishing characters given by all other investigators cited in this paper are partially incorrect or, at best, incomplete and misleading. This misleading information has led to incorrect identification of species as illustrated in several of the studies recorded herein.

It was not found possible in these studies to distinguish between physiologic forms of U. avenae or U. levis by means of any spore or panicle characters. It was possible, however, to differentiate some of them by means of their cultural characters on agar media. In the limited cultural studies conducted it appears that Medium No. 7 of Ranker (24) was the best for this purpose. It seems, however, that one is not justified in assuming that every variation on a culture medium is a physiologic form capable of infecting oats. It might be, in some cases, a single sporidial culture as indicated by Dickinson (9).

SUMMARY

1. Incorrectly labeled specimens of U. avenae and U. levis are common in the herbarium of the Department of Botany and Plant Pathology.

2. Investigators at various experiment stations in the several states are unable to identify U. avenae and U. levis in all cases by means of panicle characters.

3. The only reliable way to separate the two species is by the presence or absence of echinulations on the spore walls. These can be detected only with high magnification.

4. A whitish membrane over the spore masses is almost always present in U. levis and absent in U. avenae.

5. The amount of visible smut or the portion of the glumes affected is wholly unreliable as a means of distinguishing the two species.

6. There are no consistent or easily recognized morphological differences between physiologic forms of the same species of oat smut, or between oat varieties infected with the same physiologic form. As a rule there are no differences.

7. U. avenae is apparently the more prevalent species in the southern Great Plains, judging from the fact that 13 of the 15 collections taken in Kansas, Oklahoma and Texas and grown at the Kansas Agricultural Experiment Station are of that species.

8. The southern form of oat smut to which Kanota and Fulghum are highly susceptible is U. avenae.

9. At least some physiologic forms of U. avenae can be distinguished by cultural characters on certain media.

10. Cultural characters indicate that the collection taken at Paola, Kansas, in 1929 is a distinct physiologic form.

11. Ranker's medium No. 7 seems to be a satisfactory synthetic medium for culturing oat smuts.

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Plate I. Panicles 1-5. Types of panicles found in same smut collection on same variety of oats, Kanota (No. 2 is aberrant form). Panicles 6-7. Typical covered smut.

1
2
3
4
5
6
7

Plate II. Two collections of covered smut on vegetable agar media.

Row 1 - potato dextrose.

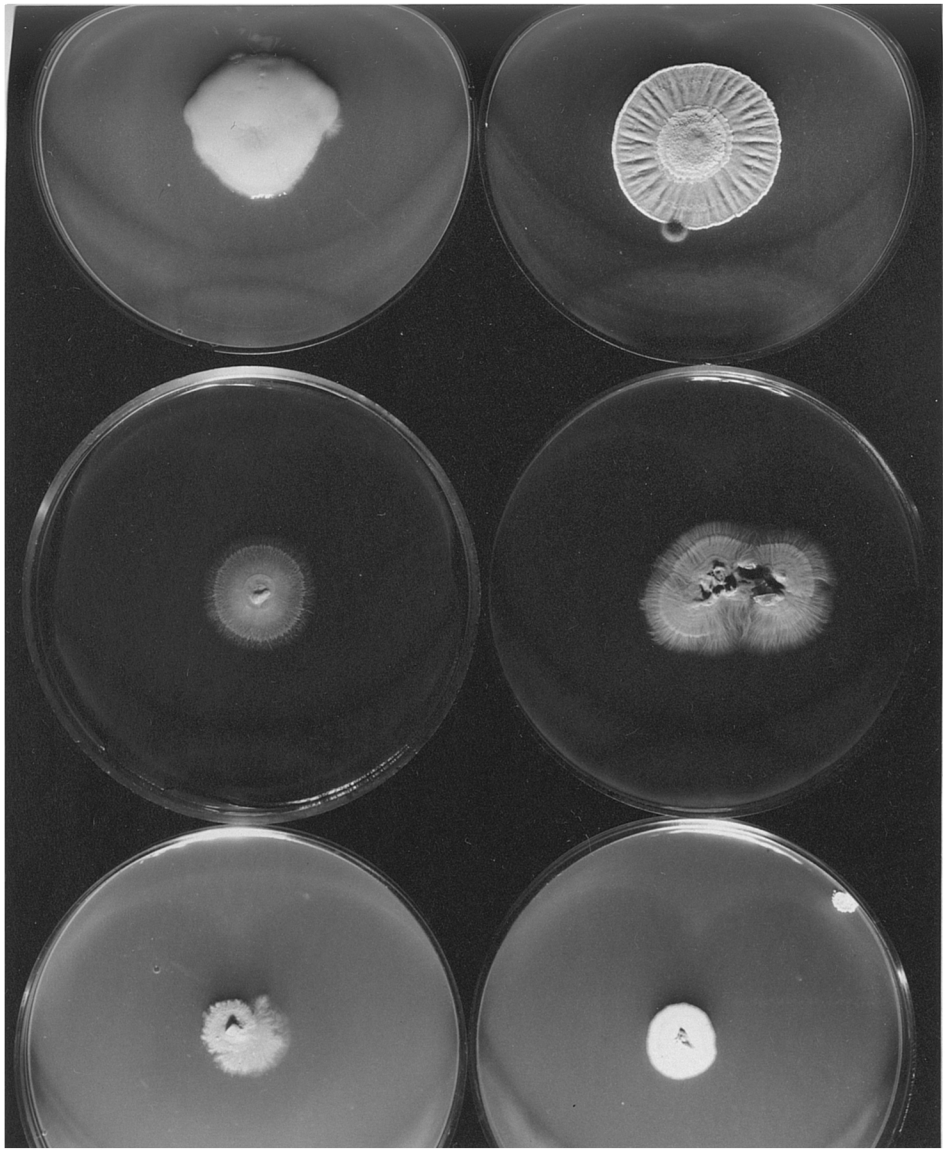
Row 2 - carrot.

Row 3 - oat.

Row 1.

Row 2.

Row 3.



C_1 C_2

Plate III. Three collections of loose smut of oats on vegetable agar media.

Row 1 - potato dextrose.

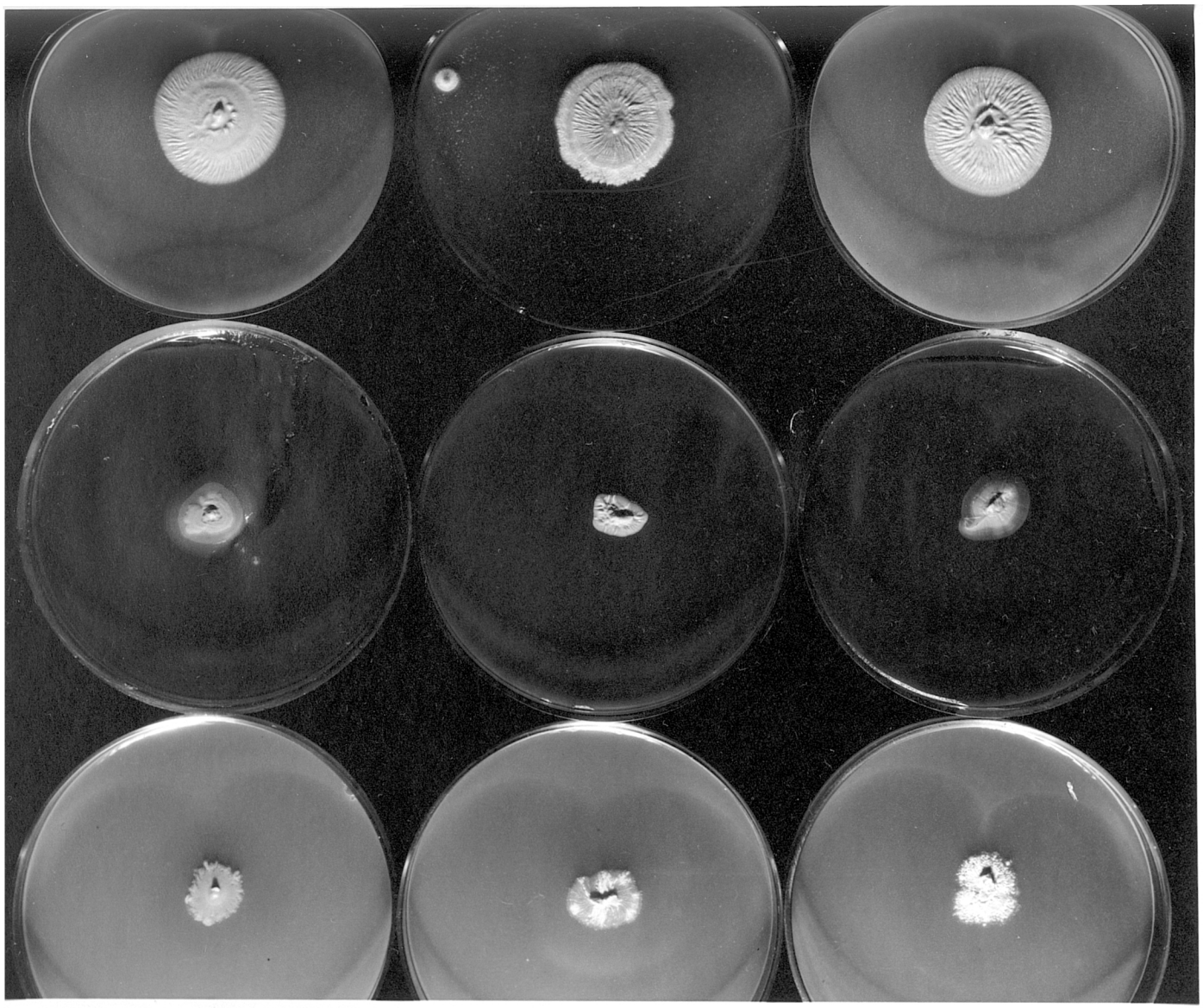
Row 2 - carrot.

Row 3 - oat.

Row 1.

Row 2.

Row 3.



L_1 L_2 L_3

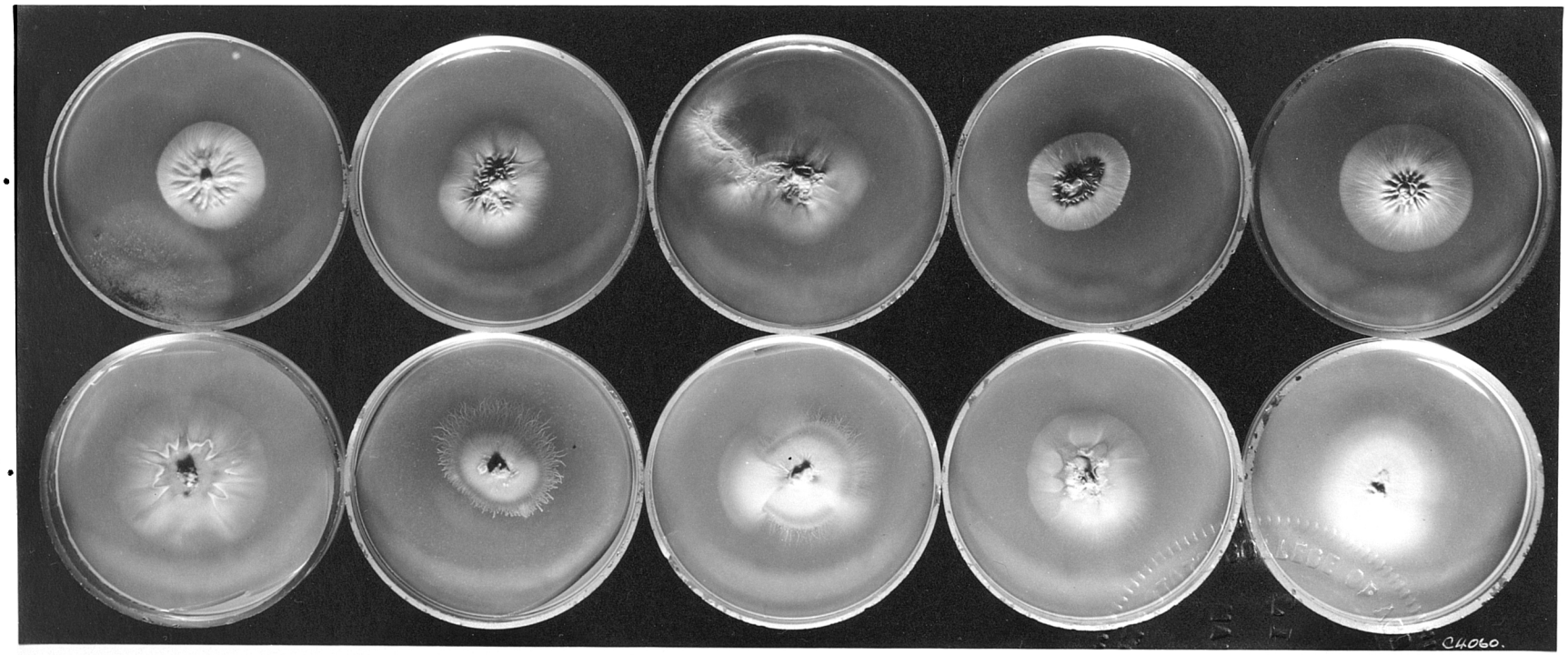
Plate IV. Two collections of covered smut and three of loose smut on synthetic media.

Row 1 - Ranker's No. 7.

Row 2 - Ranker's No. 12.

Row 1.

Row 2.



C_1 C_2 L_1 L_2 L_3