A STUDY OF PHYSIOLOGIC SPECIALIZATION OF *TILLETIA LAEVIS* IN KANSAS

by

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INTRODUCTION

Bunt of wheat, Tilletia laevis Kühn and Tilletia tritici (Bjerk.) Winter, has been ranked by Gaines (10) as the most destructive cereal disease in the United States. The average annual reduction in yield for Kansas alone due to bunt over a ten-year period from 1918 to 1927 was 5,007,000 bushels (41).

Records do not indicate that bunt was a problem in Kansas 15 or 18 years ago. The increase in loss from bunt in recent years, however, has been tremendous. This is true not only for Kansas but also for the United States as a whole.

The statement is frequently heard among farmers, that bunt in Kansas was not a problem until Kanred, a selection from Crimean wheat, became established. Kanred is thought to be essentially similar to Turkey, both morphologically and physiologically. Its reaction to bunt, therefore, would be expected to be similar. On the contrary, it has proved highly susceptible to bunt in Kansas, while Turkey retains its resistance. Blackhull, another Turkey strain, is grown extensively in Kansas and is found to be as susceptible as Kanred.

In the light of our present knowledge of pathogenic fungi and their physiologic behavior, the question has arisen, as to whether such outbreaks of bunt as have occurred in Kansas in recent years might not be largely attributed to the occurrence of more virulent strains of the bunt organism. To answer this question, tests have

been conducted in the Nursery of the Department of Botany and Plant Pathology at Manhattan, Kansas, over a three-year period (1928-1931, inclusive) to determine the reaction of 16 differential varieties of wheat to 36 collections of bunt from different localities in Kansas. In addition, laboratory studies of the smut fungus were conducted to serve as a check on varietal reactions and possibly substantiate field results.

It is the purpose of this thesis to present the results of these investigations and to briefly review the literature pertaining to physiologic specialization in the cereal smuts, placing special emphasis on the specialization in Tilletia laevis and T. tritici, the two organisms causing bunt of wheat. A study of the bunt problem from this point of view should provide fundamental knowledge for the problem of breeding for resistance to bunt of wheat in Kansas.

REVIEW OF LITERATURE

The literature pertaining to physiologic specialization of parasitic fungi is extensive. The phenomenon has been widely investigated both in the United States and in other countries, numerous species of fungi having come under observation and study.

It has been stated by Reed (20) and Rodenhiser (29) that the discovery of physiologic specialization in fungi pathogenic to plants is one of the most important developments in the science of plant pathology in recent years. According to Reed (20) the phenomenon was first suggested by Schroeter in connection with his work on a rust of Carex, in which he employed the term "sister species."

Early investigations of physiologic specialization were largely confined to the determination of differences in pathogenicity to certain species and genera of plants and the host range of the various parasitic races. In connection with this type of investigation, Eriksson (6) first called attention definitely to the nature of biologic specialization. He showed that the stem rust of cereals and grasses, Puccinia graminis, although the same morphologic species wherever found, may constitute one form upon one host species and other forms upon other host species, as for example the stem rust of wheat and the corresponding stem rusts of oats and rye. These physiologic entities were spoken of as parasitic races.

According to Reed (20), Neger did the initial work in ascertaining whether specialization occurred among the species of the powdery mildews. The most accurate work in

this regard in the powdery mildews has been done with Erysiphe graminis, the species attacking cereals.

Stakman and Piemeisel (37) in 1917 first showed that biologic specialization may occur within a fungous race as evidenced by the appearance of a component form within Puccinia graminis tritici, distinguished by its pathogenicity on varieties within a species of wheat. This new category in the classification of parasitic fungi has been greatly extended, especially in the rusts of cereals and grasses.

Prior to 1919, the smuts as a group were largely neglected in the investigation of physiologic specialization. Kneip (14a) in 1919 first demonstrated the presence of physiologic forms in the smut fungi by showing differences in strains of Ustilago violaceae grown on artificial media. Zillig (43) later found that the physiologic forms of this same fungus could be differentiated pathogenically.

Outside of certain verifications of these discoveries, little information on specialization in the smuts appeared before 1924. Considerable evidence, however, pointed to the existence of specialization in various cereal smuts, and in 1924 Faris (8) definitely demonstrated the presence of physiologic forms in Ustilago hordei, the covered smut of barley.

Reed (21), in testing strains and varieties of the various oat groups for their reaction to collections of Ustilago avenae and U. levis from Missouri, obtained results which did not in all respects agree with the results from similar tests conducted by Sampson and Davis (34) with loose and covered smut collections from Wales. This discrepancy suggested the possibility of physiologic specialization in the oat smut organisms. Furthermore, reports showed the occurrence in various southern localities of smuts on the resistant Red Rustproof and Fulghum oats. Reed, therefore, planned definite investigations on physiologic specialization in the two oat smut species and from the data obtained (22, 23) there was distinct evidence of specialization. Avena brevis Roth., consistent in its marked resistance to Ustilago levis from Missouri, "broke down" to give 100 per cent infection with the covered smut from Wales. The varieties Canadian, Potato, Victor and Monarch showed practically 100 per cent infection with the Missouri collection of U. levis but no infection from the Wales collection. The oat varieties Red Rustproof and Fulghum proved consistently resistant to Missouri collections of loose smut of oats in Reed's varietal tests at Brooklyn. Three collections of loose smut of oats were obtained by Reed (23) from Oklahoma, Texas, and Tennessee

and tested on differential varieties at Brooklyn. Red Rustproof gave practically negative results with these smut collections, while Fulghum was severely smutted. Monarch was moderately susceptible. Markton remained practically free from infection.

A collection of loose smut on Red Rustproof from Texas was also tested for its pathogenicity (23). It did not infect Fulghum but severely attacked Red Rustproof. Two separate forms of Ustilago avenae were, therefore, clearly separated, one attacking Fulghum and the other Red Rustproof.

Unpublished data by members of the Botany Department of the Kansas State College of Agriculture and Applied Science on physiologic specialization in oats smut collections from various localities in Kansas, Oklahoma, and Texas, indicate the presence of at least two forms of loose smut of oats in this region, namely, a common form comparable to Reed's Missouri form of Ustilago avenae and the so-called "southern form" attacking Kanota and Fulghum oats.

In a study of varietal resistance to oat smuts in Wales, Miss Sampson (33) demonstrated by their infection capacities, four physiologic forms of Ustilago avenae and two of U. levis. One form of U. avenae and another of U.

levis from Wales are described by her for the first time.

Reed (24) later reported on physiologic specialization in fifteen different collections of loose smut, and nine of covered smut of oats from widely separated localities. He was able to distinguish eleven strains of Ustilago avenae and five of U. levis by reaction in the field.

Tisdale, Melchers, and Clemmer (39) studied the pathogenicity of two strains of covered kernel smut of sorghum which they designated as common and milo strains. They gave evidence of a third strain attacking feterita and certain of its relatives.

Further studies on physiologic specialization in kernel smut of sorghum were made by Melchers, Ficke, and Johnston (17). Eighty varieties, selections, and hybrids of sorghum were grown to test their reaction to strains of kernel smut. Three physiologic forms of Sphacelotheca sorghi were distinguished, two corresponding to those previously reported (39) and a third attacking feterita.

Christensen and Stakman (4) separated fifteen forms of corn smut, Ustilago zeae, by their manner of growth on artificial media. They later differentiated seven, and possibly eight of these fifteen forms by the reaction on ten selfed lines of corn.

Rodenhiser (28) distinguished three distinct physiologic forms of Ustilago tritici and six of U. nuda. These forms were from widely separated localities.

Varietal differences in resistance of wheat to bunt, Tilletia tritici and T. laevis, were perhaps noted long before any written statement was made on the subject. Considerable attention has been given to bunt resistance in wheat for thirty years or more. It has been emphasized more especially since the beginning of breeding for resistance. Varietal testing and breeding for resistance to bunt have perhaps received greatest attention in the North and Northwest where seed treatments are not entirely satisfactory because of soil infestation.

As early as 1909 Pye (19) showed that all wheats grown at that time in Victoria were more or less subject to bunt infection, but the hardier varieties tended to show smaller percentages.

Reed (25) at the Brooklyn Botanical Gardens and Tisdale, Martin, et al. (40) performed extensive tests, including most of the commercial varieties of wheat grown in this country, as well as pure line selections from these varieties. They found bunt infection to vary from 0 to 97 per cent. Twenty or more varieties proved highly resistant.

Stakman, Lambert, and Flor (38) concluded from their studies that the durum wheats as a class were more resistant to bunt, Tilletia laevis, than common wheats, but that there were wide differences in both classes.

McAlpine (16), Kirchner (14), Sampson (34), Limbourn (15), Roeimer (31), Reichert (27), Gaines (9), Arnaud (1), Conners (5), Schafer (36), Nieves (18), Brentzel and Smith (2), Bressman (3), and others, present further results on species and varietal reaction of wheat to bunt. In the main, their results indicate more general resistance among the durums than among the common wheats. Winter wheats, as a rule, are more resistant to bunt than spring wheats. Durums are much more susceptible to Tilletia tritici than to T. laevis. Certain varieties, such as Genoa, Cologna, Odessa, Turkey, Ridit, Hussar, Martin, Florence, Bunyip, Hohenheimer, Minturki, Oro, and others, stand out as significantly resistant to or immune from the collections or forms of bunt used.

Faris (7), in his studies on the influence of various factors on infection of wheat by Tilletia laevis and T. tritici, obtained evidence of a difference in infection by bunt from different localities. His object was not to distinguish physiologic forms. He tested six collections

of each species of bunt on ten different varieties of winter wheat. The most definite evidence of specialization was observed in the reaction of Kanred to certain collections of T. tritici.

Miss Sampson (32) concluded from her studies of varietal reaction, that bunt, Tilletia tritici, in Wales was not a fungous organism comprising many physiologic forms.

A severe outbreak of bunt, T. laevis, in 1925 in the hard red spring wheat region of the United States suggested the presence of unusually virulent strains of the pathogen in that region. This, together with preliminary evidence of differences in pathogenicity reported by Faris (7), led Rodenhiser and Stakman (30) to plan definite studies on physiologic specialization in T. laevis and T. tritici. These investigators succeeded in showing that both species of bunt contain physiologic forms as recognized by their degree of virulence on the wheat varieties, Kota C.I. 5878, Marquis C.I. 3641, and Einkorn C.I. 2433.

Collections of bunt were made from widely separated localities. Three, and possibly four, forms of T. laevis were isolated; one from Minnesota, one from Hungary, and one from Egypt, with possibly a fourth from Italy. Two forms of T. tritici were recognized; one from Norway and

one from New Zealand. The authors suggest the possibility of a number of forms in the two species of bunt if the right differential hosts can be selected to differentiate them.

In 1927 bunt appeared on hitherto immune strains of wheat at five different stations in Washington, Oregon, and Montana. Gaines (11) attributed this and the gradual increase of bunt in recent years in such states as Kansas, Virginia, and Pennsylvania to new physiologic forms. An experiment planned by Gaines (10) to test the comparative pathogenicity of T. tritici from Germany, with that common in eastern Washington, showed distinct differences. American wheats were susceptible to the German form of bunt, while the German wheats succumbed more readily to the American form. The author distinguishes five forms of bunt on Turkey wheat. He believes Tilletia tritici has at least three forms, one form from Pullman, Washington, another one from North Dakota, and a third from Germany. Tilletia laevis contained the old form and the eastern Washington form characterized by 10 and 75 per cent infection respectively on Turkey.

Roeimer in Germany (31) grew American and German varieties of wheat inoculated with bunt from the United States and from different places in Germany and Europe. He used

as differentials, three resistant and two susceptible German wheats, five resistant American wheats, and three hybrid populations of a resistant by susceptible cross. Thirteen varieties were inoculated with fifteen collections of bunt. In general, his findings are in agreement with those of Gaines (10). Roeimer does not attempt to estimate the number of physiologic forms in his bunt collections. He suggests that each collection might be a mixture of several pure lines.

Reichert (27) found immunity and marked resistance respectively in the varieties Florence and Bunyip in Palestine. He states, "This is contrary to their habit in the countries of their origin and may be explained by the existence of different physiologic forms of bunt, or of resistant strains of wheat within the variety."

Reed (26) reports on extensive pathogenicity tests conducted from 1924 to 1927. Differences in behavior of Kanred, Turkey, Hussar, Martin, and Odessa to the smut collections made, indicate the presence of at least four physiologic forms of T. laevis and six of T. tritici. According to him, form I of T. laevis from Czecho Slovakia is characterized by its vigorous infection on Martin, Turkey, and Odessa, with slight infection on Hussar and Kanred. Form II from France severely attacked Kanred,

Turkey, Hussar, Martin, and Odessa. Form III from Missouri severely attacked Kanred and Turkey (717). Martin, Turkey (729), Hussar, and Odessa remained practically free from bunt. Form IV from New York infected Kanred and Turkey (717) but not Turkey (729), Hussar, Martin, or Odessa.

Form I of T. tritici from Czecho Slovakia severely infected Hussar, Martin, Odessa, Kanred and Turkey. Form II from New York attacked Kanred, Odessa, Turkey, and Hussar, but in 1926 and 1927 produced no infection in Martin. Form III from Czecho Slovakia attacked Kanred and Turkey (717). Turkey (729), Hussar, Martin, and Odessa were highly resistant. Form IV from New York severely attacked Kanred and Turkey (717) and occasionally Odessa and Hussar, but not Martin. Form V from Vienna attacked Kanred and Turkey (717) but not Martin and Turkey (729). Form VI from England and Wales gave negative results on Hussar, Martin, and Odessa with almost no infection on Kanred and Turkey.

Holton (12) reported on the reaction of five collections of bunt from Washington, California, Manitoba, North Dakota, and Minnesota. The first three collections were equal in virulence on the differential varieties used. The collection from North Dakota severely infected the durum wheats, Mindum and Pentad. Bunt from Minnesota attacked

Vernal Emmer. Three forms of bunt are thus distinguished.

Bressman (3) in 1931 reviews the literature on varietal reactions to bunt and physiologic specialization in the two bunt species. His studies on physiologic specialization reveal at least ten forms of bunt in 94 collections from numerous sources in Oregon and other states in the United States. Six of these are in the species Tilletia laevis and four in T. tritici. These forms are distinguished by their capacity for infection on the wheat varieties Albit, Hussar, Ridit, and Oro, and are numbered I to X.

The author states that T. laevis is common east of the Rocky Mountains while T. tritici is the predominant species west of the mountains. His trials, however, indicate no real significance to this distribution, or, in fact, to differences between species. He believes that form differences are more significant than species differences.

Little study has been made of the cultural habits of wheat bunt on artificial media, and such results as have been obtained are conflicting and inconclusive in many respects.

The first account of growing bunt on solid medium is given by Sartoris (35).

Studies on the culturing of strains of bunt on artificial media have been reported by Kienholz and Heald (13). These investigators found potato 4-per cent sucrose agar the best for differentiating strains of wheat bunt in culture. Oatmeal 3-per cent dextrose agar and Sartoris's synthetic medium also proved useful in this regard. Preliminary mass-spore cultures of the bunt collections used in their studies yielded five distinct forms of each species distinguished by size, color, and consistency of the cultures, colony characters, and color of medium.

It is evident from the foregoing investigations that physiologic specialization is common among the cereal smuts, and is characteristic of both species of wheat bunt. It seems probable that only a fair beginning has been made on the isolation of physiologic forms of Tilletia tritici and T. laevis. No exhaustive experiments have been made for the selection of differential hosts. A limited study only has been made on distinguishing strains of bunt by cultural characters on artificial media. The possibility of securing strains of bunt has not been extensively exploited, considering the possibility that different forms of smut may be found in the same locality, in the same field, in the same head of wheat, or even in the same smut ball. The unusual increase in severity of bunt in certain regions

of this country in recent years may possibly be explained through the introduction of new physiologic forms and by the constant increase in number of forms due to hybridization among them and to mutations. The problem of physiologic specialization in the two bunt species may ultimately prove more complex than investigations thus far would indicate.

OBJECTS OF INVESTIGATIONS

1. To determine the reaction of a selected group of differential varieties of winter wheat to collections of bunt from various wheat growing sections in Kansas.

2. To ascertain through their infection capacities the nature and number of pathogenic strains represented in the various Kansas collections of bunt.

3. To study the cultural characters of the apparently distinct pathogenic strains on artificial media, to determine if differences might substantiate field determinations.

4. To determine, as a practical consideration in future programs of breeding for bunt resistance, the distribution of the physiologic forms of bunt in Kansas.

MATERIALS AND METHODS IN FIELD STUDIES

On the suggestion of Mr. C. O. Johnston, Associate pathologist of the Division of Cereal Crops and Diseases, U. S. D. A., 15 collections of wheat bunt were secured for inoculum in the autumn of 1928 from 12 different counties in Kansas. The sources of these collections are indicated on the map in Figure 1. Some of the collections of bunt were in the form of threshed grain containing few or many bunt balls, while others contained unthreshed bunted heads. All collections, however, came from the 1928 wheat crop and were taken at random from field or bin. Wherever possible, the variety of wheat on which the bunt occurred was ascertained and the estimated percentage of infection recorded.

From the outset, precautions were taken to prevent the mixing of the spores of one collection of bunt with those of another. In the preparation of the inoculum, smut balls were removed from but one collection at a time. The operation for each collection was performed in a different location in the Botany building or greenhouse. Measures were taken to have all instruments, table tops, and bottles for inoculum, clean and sterile. The bunted heads or bunted grain of a collection were first placed on a clean piece of white paper. Smut balls were then removed by

means of tweezers and dropped into a screw-top bottle. The smut balls were then crushed in the bottle by means of a clean wooden pot label.

Grain of the differential varieties of wheat was first treated in bulk with formalin 1-320 solution for thirty minutes to insure bunt-free seed. The grain was then rinsed in running water and dried by spreading on sheets of clean blotting paper. Seed from the various varieties was weighed out in five-gram packets for planting and arranged in as many sets of differentials as there were collections of bunt inoculum. Each set of differential varieties was then inoculated with a collection of bunt by shaking the grain of each variety with a weighed amount of smut. A separate screw-top bottle was used for inoculating each set of varieties. Each operation was performed in a separate place in the Horticulture Building or Botany greenhouse. Drafts which might have blown spores from place to place were avoided.

The 15 sets of differential varieties were sown in series and in rows five feet long and one foot apart. Sowing operations were planned so all seed could be sown on the same day. A soil temperature suitable for optimum bunt infection was used, 35 to 60° F.

precautions were also taken to prevent mixing of bunt spores among the bunt collections in the sowing operations. Rows were opened for but one set of differential varieties at a time. Hands were washed with soap before the sowing of each set of wheat varieties. The flaps of the seed packets were opened by removing the clip with the left hand and pressing the packet edgewise between the thumb and fingers of the right hand. In this way, the fingers did not come in contact with the smutted grain. In spreading the seed, the mouth of the packet was held very close to the ground to avoid wind currents. The seed of each set of varieties was then covered and tramped before the sowing of the succeeding set.

The first row of each set of differential varieties was labeled as to source of bunt. Variety labels were necessary for the first set only, the variety arrangement being the same for all succeeding sets.

At harvesting time each row of wheat was cut by hand with a sickle and tied in a bundle and labeled. But one set of differential varieties was cut at a time. Care was always taken in cutting to prevent the catching of broken or leaning culms from the adjoining row. Counts were made by means of a hand tabulator, the bunted heads being removed and counted first. Less chance for error was ex-

perienced in the use of a mechanical hand tabulator than by "mental" counting. Partially smutted heads were counted as smutted. In case of doubt as to the presence of bunt, the head was clipped with scissors at various intervals from apex to base to expose the spore masses. The counting of bunted heads was delayed until the grain began to ripen, in order that seed of the varieties might be saved for sowing and matured smut balls collected for inoculum. The bunted heads from each set of differential varieties were placed in large paper bags. These bags were labeled individually as to variety of wheat and source of bunt, and stored for future use.

Preparations for and actual sowing operations for each successive season were essentially the same as those for the fall of 1928. Other operations involved in harvesting and counting the bunted heads were also similar.

It was possible, after the first year's tests, to eliminate from the selected differential varieties certain ones of little promise as differential hosts.

DISCUSSION OF RESULTS

Environmental conditions in the fall of 1928 were very favorable for bunt infection. Soil moisture at planting was 22.2 per cent of the moisture-holding capacity. The mean

soil temperature for the week after sowing was 56.1° F., the average minimum 53° F. and the average maximum 59.2° F.

Table I shows the reactions of 16 varieties of winter wheat to 15 collections of bunt from different localities in Kansas in 1928. The varieties are arranged from left to right in the same order as they were sown in differential series in the nursery. Cereal Investigation numbers are used in the first table only. For convenience and simplicity they are omitted from subsequent tables and the discussion of results. Beneath each variety of wheat appear its reactions to the various collections of bunt expressed in percentage of bunted heads. Usually the total number of heads per row numbered from 200 to 475.

It will be observed in Table I, that the varieties Kanred, Blackhull, Superhard, Kawvale, and Fulhard react similarly, in that they are very susceptible to all collections of bunt. All of these varieties with the exception of Kanred were omitted from succeeding tests because of their marked susceptibility.

Table I.--Reaction of varieties of winter wheat to bunt collections from different parts of Kansas. Manhattan, Kansas, 1929.

Source of smut, 1928	Per cent smut																							
	Turkey C.I. 1558-4	Kanred C.I. 5146	Hussar C.I. 4843	Martin C.I. 4463	White Odessa C.I. 4655	Blackhull C.I. 6251	Oro C.I. 8220	Turkey X Bearded Minn. #48 C.I. 8243	Minturki X Bel. Buf. C.I. 8033	Ridit C.I. 6703	Banner Berkeley C.I. 7362	Regal C.I. 7364	Superhard C.I. 8054	Cooperator-ka C.I. 8861	Kawvale C.I. 8180	Fulhard C.I. 8257							
Not smutted	*0.18	0.0	0.26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0							
Barton County (on Blackhull 15%)	0.18	61.94	0.0	0.0	0.23	74.64	3.08	0.0	2.79	5.68	0.0	0.42	69.47	0.42	63.03	55.47							
Barton County (on Turkey 1%)	0.0	79.71	0.0	0.0	0.0	72.75	0.6	0.0	2.2	11.19	0.0	2.46	13.23	1.53	83.04	67.96							
Clark County (on Blackhull 12%)	0.0	41.55	0.0	0.0	0.26	57.52	2.39	0.0	4.47	10.39	0.0	0.2	89.68	2.28	78.18	74.06							
Comanche County	0.8	60.39	0.0	0.0	0.0	81.35	0.59	0.0	1.29	5.05	0.0	2.08	67.59	5.62	68.89	73.43							
Ford County (on Turkey 24.8%)	0.75	73.31	0.0	0.0	0.0	63.36	2.96	0.0	0.8	3.57	0.0	1.8	57.84	1.39	56.34	43.06							
Gray County (on Turkey 3%)	0.44	60.33	0.0	0.0	0.0	44.05	1.22	0.0	0.75	1.36	0.0	0.0	72.4	1.84	41.17	59.56							
Gray County (on Blackhull 8%)	0.0	68.3	0.87	0.0	0.0	65.64	3.03	0.0	0.0	2.35	0.0	0.68	76.71	2.68	55.9	65.6							
Johnson County (on Harvest Queen 7%)	0.6	49.89	0.0	5.73	6.87	71.48	0.65	0.0	1.4	0.0	34.38	5.5	76.92	15.29	65.26	77.97							
McPherson County (on Blackhull 10%)	0.6	85.46	0.0	0.0	0.0	84.44	3.09	0.0	5.89	0.0	0.0	1.2	66.10	1.83	84.89	80.16							
Meade County (trace on Blackhull)	0.23	77.82	0.0	0.0	0.0	72.45	1.83	0.0	3.4	1.53	0.0	2.29	78.61	1.51	89.22	75.84							
Ness County (on Blackhull)	1.4	72.13	0.0	0.0	2.2	57.22	0.0	0.2	2.59	0.0	0.22	3.69	71.57	6.5	67.19	77.8							
Pawnee County (on Blackhull 27%)	6.2	72.31	0.0	0.0	0.0	47.21	0.0	0.8	2.78	0.51	0.24	0.7	84.8	0.64	57.51	68.83							
Reno County (on Blackhull)	6.63	60.46	0.0	0.0	0.0	62.03	0.86	0.0	2.98	0.0	0.0	0.0	58.14	0.0	56.21	78.36							

Pratt County (on Blackhull 17%)	14.4	53.38	0.0	0.0	0.0	46.93	0.0	0.0	0.0	0.0	0.0	0.0	36.84	1.55	25.86	60.71
Thomas County (on Prelude)	0.0	41.39	0.0	0.0	0.0	41.5	0.0	0.31	2.09	0.0	4.04	1.4	32.83	0.0	27.05	48.33

*The data in this table are based upon plantings made by Mr. A. T. Bartel in the fall of 1928. His records show that seed inoculated with bunt collections were pretreated with formalin. The uninoculated seed evidently was not pretreated. The small percentages of bunt in Turkey and Hussar may be due to off-type plants in the row or to stray heads caught from an adjoining row.

Martin, Hussar, White Odessa, and Turkey X Bearded Minn. #48 gave low or negative reactions to all 1928 collections of bunt.

Oro, Minturki X Bel Buf., Ridit, Regal, Banner Berkeley, and Cooperatorka showed promise as differential varieties.

Little more than mere indications of physiologic specialization can usually be detected from a single differential test of this kind. There is, of course, no way of knowing from one year's results whether a particular collection of bunt represents a single form or a mixture of two or more.

The results (Table I) indicate the presence in Kansas of at least two, and possibly more, physiologic forms of bunt. First is the common form to which the varieties Kanred, Blackhull, Superhard, Kawvale, and Fulhard are all very susceptible, and the varieties Turkey, Hussar, Martin, White Odessa, Oro, Turkey X Bearded Minnesota #48, Minturki X Bel Buf., Ridit, Banner Berkeley, Regal, and Cooperatorka are significantly resistant. This form, temporarily designated as Form I, is represented in the 1928 bunt collections from Barton, Clark, Comanche, Ford, Gray, McPherson, Meade, and Ness Counties.

The collection of bunt from Johnson County (1928) differs from all others in its high infection on Banner Berkeley (34.38%) and moderately high infection on Cooperatorka (15.29%). This collection will be temporarily designated as Form II.

A possible third form occurs in the bunt collections from Pawnee, Reno, and Pratt Counties. The infection range is from 6 to 14 per cent on Turkey.

Unfortunately, through an error or a misdirection, bunted heads from the various sets of differential hosts in the 1929 crop were not saved for inoculum. It became necessary, therefore, again to send afield for bunt collections in the autumn of 1929. These collections were obtained in much the same manner as were the 1928 collections, the sources being well distributed over the central, western and northwestern sections of Kansas as shown on the map in Figure 1. In some instances bunt was secured from the same counties in which collections had been made the previous year.

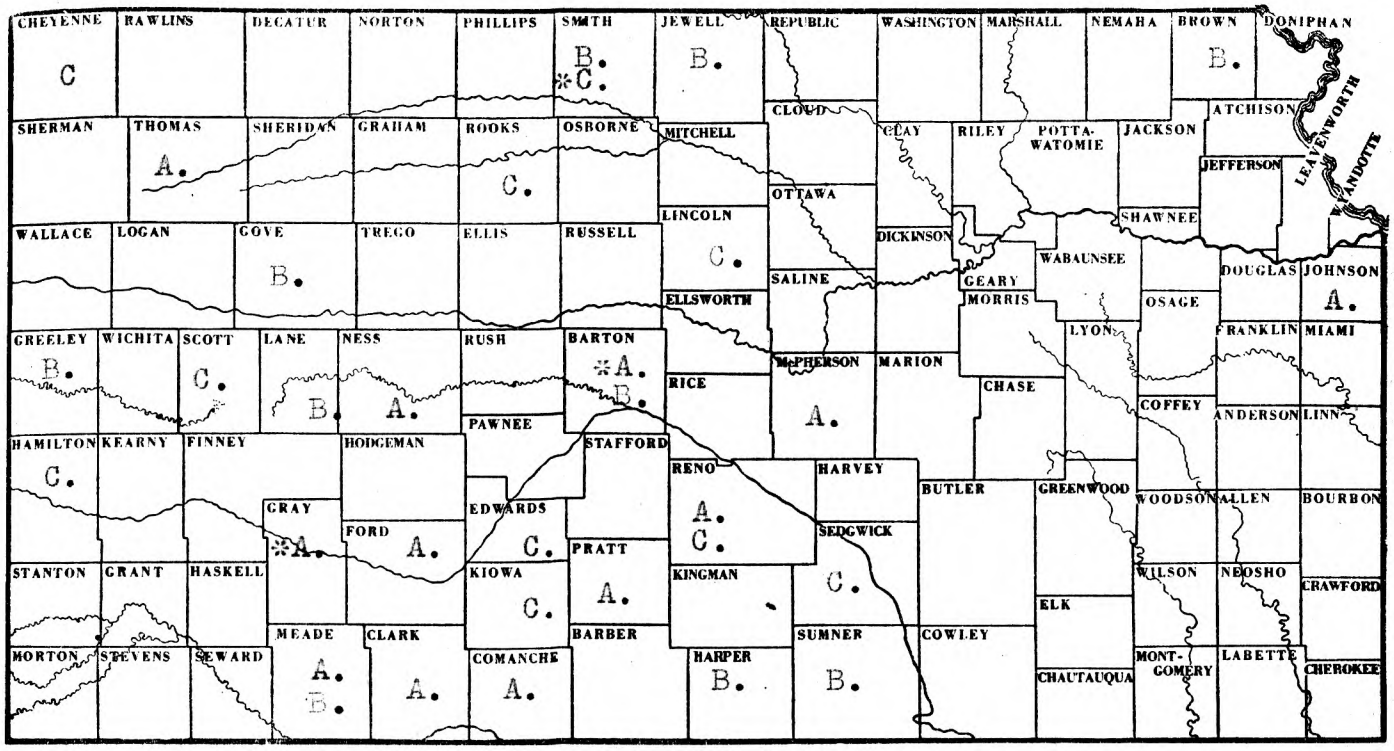


Fig. 1. Sources of bunt collections.
A. 1928 collections.
B. 1929 collections.
C. 1930 collections.
* Two collections made in same year.

It was generally observed in all experiments involving wheat bunt infection at Manhattan for the season 1929-1930, that bunt had for some reason not been seasonably favored. Infection percentages were low, even in the more susceptible varieties of wheat. The differential nursery was sown October 23, 1929. The mean soil temperature for the week following planting was 48.1° F., the average minimum 45.9° F. and the average maximum 50.3° F. The soil moisture at planting time was 25.3 per cent of the moisture-holding capacity. It will be observed in Table II that at least six of the 1929 collections of bunt from various localities in Kansas closely resemble or approximate Form I in pathogenicity. This form has been characterized as highly virulent on Kanred and giving slight or no infection on the varieties Turkey, Hussar, Martin, White Odessa, Oro, Turkey X Bearded Minnesota #48, Minturki X Bel Buf., Ridit, Regal, and Cooperatorka. The 1929 collections falling into Form I are from Jewell, Smith, Greeley, Sumner, Barton, and Gove Counties.

Table II.--Reactions of varieties of winter wheat to bunt collections from different parts of Kansas. Manhattan, Kansas, 1930.

Source of smut, 1929	Per cent smut												
	Turkey	Kanred	Hussar	Martin	White Odessa	Oro	Turkey X Bearded Minnesota #48	Minturki X Bel Buf	Ridit	Banner Berkeley	Regal	Cooperatorka
Barton County (On Kanred 1%)	0.0	4.54	0.0	0.0	0.0	0.0	2.47	0.47	0.41	0.0	0.0	1.04
Brown County (on H. Queen)	1.01	25.27	0.0	2.23	2.11	0.0	1.01	0.0	0.0	23.08	2.48	4.27
Gove County	0.0	31.57	0.0	0.0	0.9	0.0	2.46	0.64	0.0	0.0	0.0	0.98
Greeley County	0.0	22.94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.67
Harper County	4.21	34.25	0.0	0.97	0.49	0.0	0.62	1.12	0.0	0.0	0.0	0.69
Jewell County	0.33	26.92	0.0	0.0	0.0	0.38	0.31	0.0	0.0	0.0	0.0	0.0
Johnson County (1928 smut on H. Queen)	0.0	11.44	0.0	0.89	0.98	0.0	0.0	0.0	0.0	7.52	2.4	4.03
Lane County	4.09	19.14	0.0	0.0	0.0	0.0	0.0	0.0	0.45	0.0	0.0	1.6
Meade County	5.10	45.14	7.35	1.67	1.06	0.0	0.0	3.44	0.62	0.0	0.0	0.0
Smith County	0.65	33.56	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.84
Sumner County	2.12	23.75	2.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.79

A high percentage of infection on Banner Berkeley and a moderately high infection on Cooperatorka, temporarily, at least, place the collection of bunt from Brown County (1929) in Form II. Johnson County bunt, collected in 1928 on Harvest Queen and stored as inoculum until the autumn of 1929, was apparently weakened by age. Nevertheless, the collection again gave the Form II reaction with a moderately high infection on Banner Berkeley and Cooperatorka. Results thus far indicate the presence of but a single physiologic form of bunt in northeastern Kansas, namely, Form II.

The collection from Meade County (1929) may represent a new strain distinct from any found in the 1928 collections of bunt. It is significantly different in its ability to infect Hussar to the extent of 7.35 per cent and the variety Turkey 5.10 per cent.

Bunt from Harper and Lane Counties (1929) apparently belong to the group of collections previously designated as virulent on Turkey. Additional tests will be necessary to establish this point.

It was generally observed in all wheat bunt experiments at Manhattan for the season 1930-1931, that bunt was favored by environmental conditions. Bunt percentages were unusually high in the check varieties. The differen-

tial nursery was sown October 20, 1930. A second planting of varieties inoculated with 1929 bunt collections was made October 24, 1930. Varieties inoculated with collections made in 1930 were sown on the first date only. Soil moisture at the first planting was 21.73 per cent of the moisture-holding capacity and at the second planting 24.11 per cent. The mean soil temperature for the week following the first sowing was 36.20° F., the average minimum 33.1° F., and the average maximum 39.4° F. For the week following the second planting, the mean soil temperature was 36.5° F., the average minimum 34.5° F., and the average maximum 38.6° F. The same set of differential varieties used in 1929-1930 season was continued in 1930-1931. The reactions of these varieties to 11 Kansas collections of bunt tested for the second time appear in Table III. The results compare favorably with the previous year's varietal reactions. Kanred remains highly susceptible to all Kansas collections of bunt. Percentages of infection on this variety ranged from 10 to 30 per cent higher than in the 1929-1930 season. In numerous other instances the percentage of infection on other varieties was correspondingly higher.

Table III.--Reactions of varieties of winter wheat to bunt collections from different parts of Kansas. Manhattan, Kansas, 1931.

Source of smut 1929	Per cent smut												
	Turkey	Kanred	Hussar	Martin	White Odessa	Oro	Turkey X Bearded Minnesota #48	Minturki X Bel Buf.	Ridit	Banner Berkeley	Regal	Cooperatorka
Barton County (on Kanred 1%)	0.0	47.10	0.0	0.0	0.0	0.0	0.0	0.38	1.46	0.0	0.0	0.0
Brown County (on H. Queen)	0.45	32.31	0.0	19.85	21.92	0.0	0.0	0.0	0.0	57.54	8.01	19.35
Gove County	0.54	50.00	0.0	0.0	0.0	0.0	0.68	0.0	2.32	0.0	0.0	0.0
Greeley County	0.0	59.29	0.0	0.0	0.0	0.0	1.57	1.60	0.0	2.55	0.37	1.64
Harper County	0.89	36.69	0.0	6.40	4.11	0.0	0.0	0.0	2.24	4.63	0.36	3.44
Jewell County	1.62	44.00	0.0	0.0	0.0	0.0	0.31	0.0	0.70	0.0	0.0	0.0
Johnson County (1928 smut on H. Queen)	3.26	47.86	0.44	28.83	16.53	0.0	0.45	3.35	0.0	54.00	7.82	28.79
Lane County	6.40	54.38	0.0	0.0	0.0	0.0	0.34	0.60	0.46	0.0	0.0	2.17
Meade County	5.40	56.12	20.71	13.15	13.55	0.0	0.0	0.90	7.52	6.85	0.0	3.98
Smith County	2.87	51.50	0.0	0.0	0.0	0.0	0.32	1.89	0.57	0.58	0.0	1.52
Sumner County	1.31	56.27	0.0	0.0	0.0	0.0	0.0	1.59	2.32	0.0	0.0	3.21

The collections of bunt from Jewell, Smith, Greeley, Sumner, Barton, and Gove Counties again gave a Form I reaction characterized by a high infection on Kanred and low infection or negative results on all other varieties.

The bunt collections from Brown and Johnson Counties remained constant in reaction except on the varieties Martin and White Odessa. The low percentages of bunt on these varieties in 1930 may have been due to environmental conditions generally unfavorable for bunt. The higher percentages of infection in 1931 are probably due, in large degree, to a more favorable bunt season. Smut from Brown and Johnson Counties is characterized by its high infection on Banner Berkeley and its moderately high infection on Regal, Cooperatorka, White Odessa, and Martin.

Lane County smut again showed moderately high infection (6.40%) on Turkey. Harper County smut, showing 4.09% infection on Turkey in 1930, showed less than 1 per cent infection on the same variety in 1931, indicating that the former year's infection was not significant. Harper county smut, therefore, falls into Form I, characterized by its virulence on Kanred and low or negative infection on all other varieties inoculated.

Meade County smut gave a higher infection on Hussar

in 1931 than in 1930. The percentages of infection were also somewhat higher on Martin and White Odessa.

Eleven new bunt collections were tested for the first time in 1930-31. The source of these collections together with their response to 12 differential varieties are shown in Table IV.

Table IV.--Reaction of varieties of winter wheat to 1930 bunt collections from different parts of Kansas. Manhattan, Kansas, 1931.

Source of smut 1930	Per cent smut														
	Turkey	Kanred	Hussar	Martin	White Odessa	Oro	Turkey X Bearded Minnesota #48	Minturki X Bel Buf.	Ridit	Banner Berkeley	Regal	Cooperatorka
Cheyenne County	3.90	48.42	0.0	0.0	0.0	0.0	0.0	2.20	0.0	0.0	0.0	0.0
Edwards County	0.0	55.59	0.0	0.43	0.0	0.0	0.45	0.0	0.0	5.0	0.0	0.0
Hamilton County	2.88	62.32	8.10	6.23	6.67	0.95	0.71	1.47	2.85	30.35	1.02	2.79
Kiowa County	0.0	61.40	0.0	0.0	0.72	0.0	0.0	0.0	0.0	3.14	0.0	1.85
Lincoln County	0.0	67.76	0.0	0.0	0.0	0.0	0.0	2.74	2.19	0.0	0.0	3.95
Reno County	2.83	62.65	1.11	0.0	0.0	0.0	0.0	1.52	0.0	0.0	0.0	0.0
Rooks County	0.0	66.47	0.0	0.0	0.0	0.0	1.32	1.81	1.33	0.0	0.0	2.2
Sedgwick County	3.46	60.87	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Smith County (Kensington)	0.0	73.17	0.0	0.0	0.0	0.0	0.57	0.80	3.50	2.40	0.0	1.40
Smith County (Smith Center)	0.0	44.11	0.0	0.0	0.0	0.0	1.38	1.86	1.14	0.0	0.0	3.87
Scott County	7.31	70.04	1.43	0.0	0.0	0.0	0.0	0.0	0.0	5.35	1.6	3.90

The 1930 bunt collections from Cheyenne, Edwards, Kiowa, Lincoln, Reno, Rooks, Sedgwick, and Smith Counties present a Form I reaction characterized by a high infection on Kanred and low or negative infection on all other varieties inoculated.

The collection from Hamilton County differs from any bunt collection thus far tested in that it shows considerable virulence on Banner Berkeley and moderately high infections on Hussar, Martin, and White Odessa. The collection is similar in some respects to the bunt collections from Brown and Johnson Counties (Form II) but differs in showing low infection on Cooperatorka and Regal and moderately high infection on Hussar.

The bunt collection from Scott County shows moderately high infection on Turkey.

At least three distinct pathogenic strains of bunt are represented in the 1930 Kansas bunt collections.

Table V contains a summary of the reaction of 12 differential wheat varieties to 36 collections of Kansas bunt tested in the seasons 1929 to 1931, inclusive. The bunt collections have been grouped according to their several types of virulence on the differential hosts. Certain collections are represented as having been tested for two seasons. In such cases the smut percentages shown are an average of two years' results.

Table V.--Summary of varietal reactions to bunt collections from different parts of Kansas, 1929 to 1931, inclusive.

Source of smut and group	Number years collection was tested	Per cent smut: Varieties used in preparation of key: Turkey	Kussar	Martin	White Odessa	Kidit	Banner Berkeley	Cooperatorka	Other varieties included in experiments: Regal	Oro	Turkey X Bearded Minnesota #48	Minturki X Bel Buf.	Kanred
Group I													
Barton County, 1929	2	0.0	0.0	0.0	0.0	0.93	0.0	0.52	0.0	0.0	1.23	0.42	45.82
Cheyenne County, 1930	1	3.90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.20	48.42
Edwards County, 1930	1	0.0	0.0	0.43	0.0	0.0	5.0	0.0	0.0	0.0	0.45	0.0	55.59
Ford County, 1928 (on Turkey 24.8%)	1	0.75	0.0	0.0	0.0	3.57	0.0	1.39	1.81	2.96	0.0	0.80	73.31
Gove County, 1929	2	0.23	0.0	0.0	0.45	1.16	0.0	0.49	0.0	0.0	1.57	0.32	40.78
Gray County, 1928 (on Blackhull 8%)	1	0.0	0.87	0.0	0.0	2.35	0.0	2.68	0.68	3.30	0.0	0.0	68.30
Gray County, 1928 (on Turkey 3%)	1	0.44	0.0	0.0	0.0	1.36	0.0	1.84	0.0	1.22	0.0	0.75	60.33
Greeley County, 1929	2	0.0	0.0	0.0	0.0	0.0	1.27	1.15	0.18	0.0	0.78	0.80	41.07
Harper County, 1929	2	2.40	0.0	3.72	2.30	1.12	2.31	2.06	0.18	0.0	0.13	0.56	45.47
Jewell County, 1929	2	0.92	0.0	0.0	0.0	0.35	0.0	0.0	0.0	0.19	0.15	0.0	35.46
Kiowa County, 1930	1	0.0	0.0	0.0	0.72	0.0	3.14	1.85	0.0	0.0	0.0	0.0	61.40
Lincoln County, 1930	1	0.0	0.0	0.0	0.0	2.19	0.0	3.95	0.0	0.0	0.0	2.74	67.76
McPherson County, 1928 (on Blackhull 10%)	1	0.6	0.0	0.0	0.0	0.0	0.0	1.83	1.20	3.09	0.0	5.89	85.46
Meade County, 1928 (Trace on Blackhull)	1	0.23	0.0	0.0	0.0	1.53	0.0	1.51	2.29	1.83	0.0	3.40	77.82
Ness County, 1928 (on Blackhull)	1	1.40	0.0	0.0	2.20	0.0	0.22	6.52	3.69	0.0	0.20	2.59	72.13

Reno County, 1930	1	2.83	1.11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.52	62.65
Rooks County, 1930	1	0.0	0.0	0.0	0.0	1.33	0.0	2.20	0.0	0.0	1.32	1.81	66.47
Smith County, 1929	2	1.76	0.0	0.0	0.0	0.28	0.29	1.18	0.0	0.0	0.16	0.94	42.53
Smith County, 1930 (Kensington)	1	0.0	0.0	0.0	0.0	3.50	2.40	1.40	0.0	0.0	0.57	0.80	73.10
Smith County, 1930 (Smith Center)	1	0.0	0.0	0.0	0.0	1.14	0.0	3.87	0.0	0.0	1.38	1.86	44.11
Sedgwick County, 1930	1	3.46	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.87
Sumner County, 1929	2	1.21	1.07	0.0	0.0	1.16	0.0	2.00	0.0	0.0	0.0	0.79	39.96
Thomas County, 1928 (on Prelude)	1	0.0	0.0	0.0	0.0	0.0	4.04	0.0	1.40	0.0	0.31	2.09	41.39
Group II													
Brown County, 1929	2	0.76	0.0	6.04	11.51	0.0	45.31	11.81	5.24	0.0	0.50	0.0	28.79
Johnson County, 1928 (on Harvest Queen)	*2	0.63	0.22	17.28	11.70	0.0	44.19	22.04	6.66	0.32	0.22	2.37	48.87
Group III													
Meade County, 1929	2	5.25	14.03	7.41	7.30	4.07	3.42	1.99	0.0	0.0	0.0	2.67	50.63
Group IV													
Pawnee County, 1928	1	6.20	0.0	0.0	0.0	0.51	0.24	0.64	0.70	0.0	0.80	2.70	72.31
Reno County, 1928	1	6.63	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.86	0.0	2.98	60.46
Pratt County, 1928	1	14.40	0.0	0.0	0.0	0.0	0.0	1.55	0.0	0.0	0.0	0.0	53.38
Lane County, 1929	2	5.25	0.0	0.0	0.0	0.45	0.0	1.88	0.0	0.17	0.30	0.23	36.76
Scott County, 1930	1	7.31	1.43	0.0	0.0	0.0	5.35	3.90	1.61	0.0	0.0	0.0	70.04
Group V													
Barton County, 1928 (on Turkey 1)	1	0.0	0.0	0.0	0.0	11.19	0.0	1.53	2.46	0.60	0.0	2.20	79.71
Clark County, 1928	1	0.0	0.0	0.0	0.26	10.38	0.0	2.28	0.20	2.39	0.0	4.47	41.55

Comanche County, 1928	1	0.80	0.0	0.0	0.0	5.05	0.0	5.62	2.08	0.59	0.0	1.29	60.39
Barton County, 1928 (on Blackhull 15%)	1	0.18	0.0	0.0	0.23	5.68	0.0	0.42	0.42	3.08	0.0	2.79	61.94
						Group VI							
Hamilton County, 1930	1	2.88	8.10	6.23	6.67	2.85	30.35	2.79	1.02	0.95	0.71	1.47	62.32

*Percentages are an average of 1929 and 1931 results. Inoculum used in the season 1929-30 was lacking in viability.

Group I, temporarily designated above as Form I, is characterized by a high infection on Kanred and low or negative infection on all other varieties tested. Twenty-three collections of bunt are included in this group.

Group II, temporarily designated above as Form II, is characterized by high infection on Kanred and Banner Berkeley and moderately high infection on Martin, White Odessa, Regal, and Cooperatorka. Two bunt collections are included in this group.

Group III is characterized by high infection on Kanred and Hussar. Moderately high infection occurs on Turkey, Martin, and White Odessa. One bunt collection is included in the group.

Group IV is characterized by a high infection on Kanred and a moderately high infection on Turkey. Five bunt collections are included in the group.

Group V is characterized by a high infection on Kanred and a moderately high infection on Ridit. Four bunt collections are included in the group.

Group VI is characterized by high infections on Kanred and Banner Berkeley and moderately high infections on Hussar, Martin, and White Odessa. One bunt collection is included in the group.

On the basis of the grouping in Table V a dichotomous key has been prepared for the identification of the parasitic strains occurring in the Kansas collections of bunt tested in the seasons 1929 to 1931, inclusive. Six physiologic forms are differentiated by differences in pathogenicity on Turkey, Hussar, Martin, Ridit, White Odessa, and Banner Berkeley. Regal and Cooperatorka show promise as differential hosts and could possibly be included as such in this key. Other varieties, however, adequately separate the forms of bunt.

The varieties Oro, Minturki X Bel Buf., and Turkey X Bearded Minnesota #48 show resistance to all Kansas collections of bunt tested.

Key for the Identification of Physiologic Forms

Turkey C.I. 1558-A highly resistant

Hussar C.I. 4843 resistant

Martin C.I. 4463 and Banner Berkeley C.I. 7362 resistant

Ridit C.I. 6703 resistant. . . . . Form I

Ridit C.I. 6703 moderately susceptible . . . Form V

Martin C.I. 4463 susceptible, Banner Berkeley C.I. 7362 very susceptible. . . . . Form II

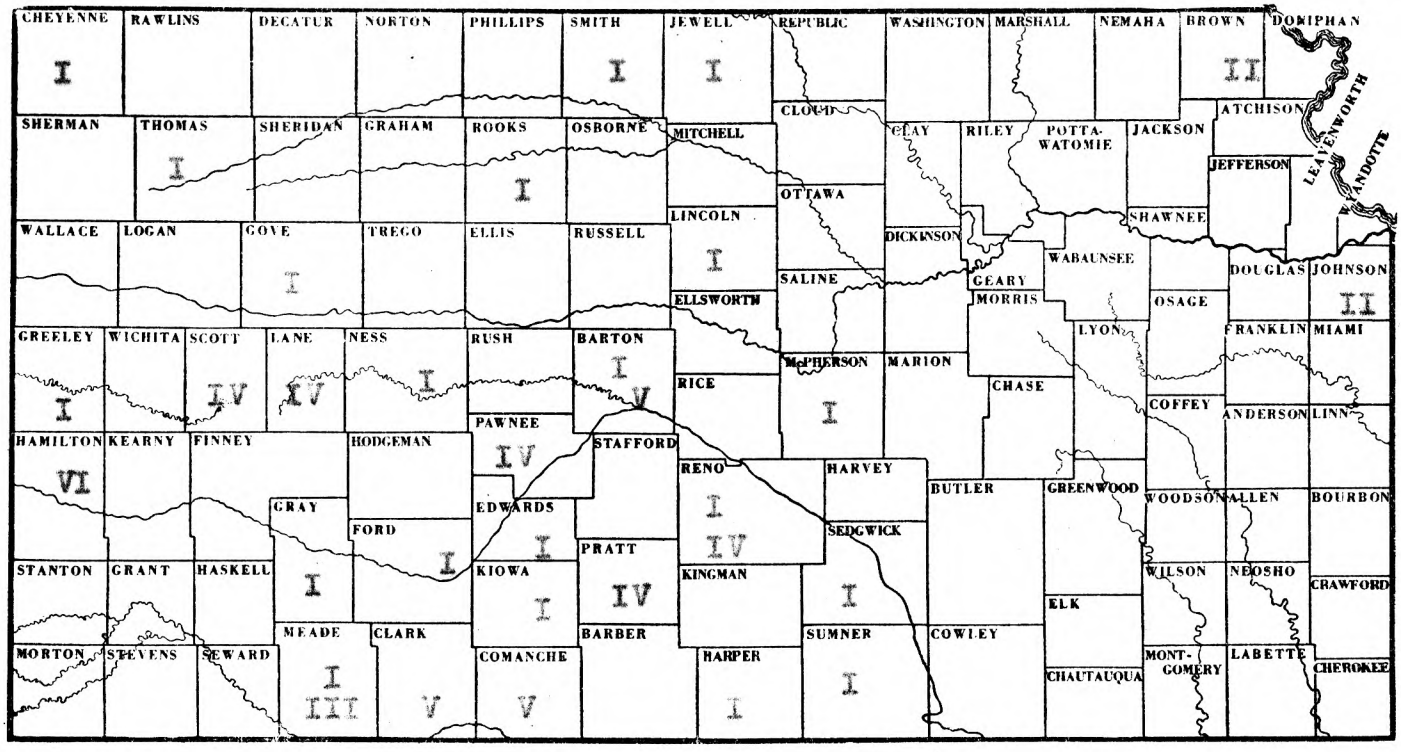
Hussar C.I. 4843 moderately susceptible. . . . Form VI

Turkey C.I. 1558-A moderately resistant

Hussar C.I. 4843, Martin C.I. 4463 and White Odessa C.I. 4655 highly resistant. . . . . Form IV

Hussar C.I. 4843, Martin C.I. 4463, and White Odessa C.I. 4655 moderately susceptible. . . . Form III

The distribution of the physiologic forms of bunt in Kansas, based on the spore collections tested, is shown in Figure 2. It will be observed, Figure 2, that Form I is apparently widely distributed over the western half of the state. Form II is confined to northeastern Kansas. Form III appears in one county in southwestern Kansas. Forms IV and V are found distributed over the south-central portion of the state.



2. Distribution of physiologic forms of bunt in Kansas.

In 1929, Weston (42) was able to "break down" the resistance of many wheat varieties such as Hussar, Sherman, Turkey, and Ridit by back-inoculating to a variety with bunt spores collected from that variety. By this method, he was able to obtain "virulent" bunt or a "pure line." It seemed interesting, therefore, to reinoculate with their own smut, certain of the differential varieties used in these experiments which showed a small percentage of infection with certain Kansas collections of bunt in 1930. The results of these back inoculations are given in Table VI. Back inoculations were not made in all cases of small infection. Frequently no more than one or two very small bunted heads or a part of a bunted head was present in a variety. In such instances inoculum was insufficient for back-inoculating. In other cases, the inoculum could not be spared, particularly Johnson County smut, in which limited infection occurred on any variety.

It will be observed, Table VI, that by back inoculation the infection on certain varieties was increased. Significant increases over 1930 infections are noted in ten cases out of thirty-two. In one case (Turkey, Lane County) the percentage was significantly reduced. No explanation can be given of the latter circumstance. The

1930 reaction on this variety is substantiated in the regular differential tests, however.

Table VI.--Varieties showing small percentages of bunt infection in 1929, reinoculated with their own smut in 1930. Manhattan, Kansas, 1931.

1930 Row No.	Variety	Source of smut 1929	Per cent smut 1930	Per cent smut 1931
107	Turkey X Bd. Minn.#48	Barton County	2.47	1.80
112	Cooperatorka	" "	1.04	0.32
116	Martin	Brown "	2.23	24.61
117	White Odessa	" "	2.11	22.40
122	Banner Berkeley	" "	23.08	57.94
123	Regal	" "	2.48	12.25
129	White Odessa	Gove "	0.90	0.0
131	Turkey X Bd. Minn.#48	" "	2.46	0.0
132	Minturki X Bel Buf.	" "	0.64	1.23
136	Cooperatorka	" "	0.98	0.0
148	Cooperatorka	Greeley "	0.67	0.0
149	Turkey	Harper "	4.21	3.63
152	Martin	" "	0.97	7.95
155	Turkey X Bd. Minn.#48	" "	0.62	0.84
156	Minturi X Bel Buf.	" "	1.12	0.0
166	Oro	Jewell "	0.38	0.0
167	Turkey X Bd. Minn.#48	" "	0.31	2.48
177	White Odessa	Johnson "	0.98	10.94
182	Banner Berkeley	" "	7.52	55.23

185	Turkey	Lane County	4.09	0.0
193	Ridit	" "	0.45	2.38
196	Cooperatorka	" "	1.60	1.72
197	Turkey	Meade "	5.10	6.02
199	Hussar	" "	7.35	37.22
200	Martin	" "	1.67	13.46
201	White Odessa	" "	1.06	21.87
204	Minturki X Bel Buf.	" "	3.44	0.88
205	Ridit	" "	0.62	10.60
220	Cooperatorka	Smith "	1.84	2.32
221	Turkey	Sumner "	2.12	3.40
223	Hussar	" "	2.15	2.29
232	Cooperatorka	" "	0.79	2.36

Increases in percentages of infection may, it is believed, be largely attributed to a more favorable bunt environment in the 1930-31 season than in the one preceding. Certain extreme increases in infection, however, tend to indicate the segregation of strains virulent on particular varieties. This condition is suggested in Brown County smut on Banner Berkeley, Martin, White Odessa, and Regal; Johnson County smut on Banner Berkeley, and White Odessa; and Meade County smut on Hussar, Martin, White Odessa, and Ridit.

Holton (11) was doubtful whether the term physiologic form could be properly applied to chlamydospore collections of bunt. The writer believes that a collection of bunt spores may constitute one or more pathogenic strains. It may be possible through continued back inoculations on varieties showing small percentages of infection to segregate a number of distinct strains from a single collection of bunt, which by continued inoculation as a mixture of spores from several differential varieties may more or less retain its original pathogenic identity.

Kansas collections of bunt, which have been tested for two consecutive seasons in these experiments, have shown considerable constancy in varietal reactions. For all

practical purposes, therefore, a collection of bunt or a group of collections showing marked pathogenic individuality may, it is believed, be designated as a physiologic form.

LABORATORY INVESTIGATIONS

Germination Studies

It seemed advisable to follow the program of Kienholz and Heald (13) and first perform some preliminary studies on chlamydospore germination and the initiation of colony growth before attempting cultural studies of the bunt collections. The desired medium was first sterilized and then poured into sterile Petri dishes and allowed to solidify. A bunt ball was then picked from a smutted head of wheat by means of tweezers and held for 2 or 3 minutes in mercuric chloride solution 1-1000. The smut ball was cut in two with a sterilized scalpel. With the point of a small scalpel, sterilized by flaming and moistened by touching it to the agar in a plate, spore masses from the center of the ball were distributed over the surface of the medium. Considerable difficulty was experienced even then, in obtaining bacteria-free plantings of certain of the bunt collections. Acidified agar was tried. While

not entirely preventing spore germination, this medium seemed not to allow the development of "white colonies" or sporidial masses suitable for transfer. Numerous attempts at sterilizing the chlamydospores by means of weak copper sulfate solutions used for varied intervals of time up to 36 hours proved unsuccessful. Germination was largely inhibited, due either to the effect of the chemical on the protoplasm of the spores or to the excess moisture necessarily introduced with the spores into the Petri dish.

It, therefore, seemed more advisable, under the circumstances, to continue making spore plantings from smut balls as first described, trusting to finally obtain a bacteria-free "colony" for transfer to other media. In this way a pure culture of a representative collection of all but one pathogenic strain of Kansas bunt was obtained.

Plates with mass-spore plantings were at first incubated at from 18 to 20° C. in one of the compartments of the constant temperature tanks in the Botany greenhouse. For some unknown reason poor results were obtained. The bunt spores germinated in greater or fewer numbers, depending largely on the medium, but "white colony" formation usually did not result.

More satisfactory results were obtained by incubating the plates at from 17 to 20° C. in a "Thelco" incubator in the Station pathological laboratory.

Chlamydospore germination tests were also performed at room temperatures. Plates with spore plantings were kept in round glass chambers into which wet filter paper was introduced to prevent the rapid drying out of the media.

The results obtained with various media at controlled temperatures and at room temperatures are described below:

Clear Agar. This medium was prepared by dissolving 20 grams of agar in 1000 cc. of distilled water and sterilizing at 15 pounds pressure for 15 minutes.

In accord with the observations of Kienholz and Heald (13), this medium proved very satisfactory for the germination of chlamydospores of Tilletia laevis. While Kienholz and Heald state that "some germination occurred at room temperature (25° C.)," germination of Greeley County bunt on this medium at room temperature occurred in from 2 to 4 days with the abundant production of uniform and regular-shaped sporidia, followed by the formation of "white colonies" in from 6 to 8 days.

At 17 to 20° C., germination and sporidial production were retarded at least 2 days, but this may have been due to light or air relations in the constant temperature chamber which were not comparable to those in the laboratory room.

Heavy Oatmeal Agar. This medium was made by boiling 100 gr. of oatmeal in 500 cc. of water and squeezing the cooked mass through a single thickness of cheese cloth. This paste-like mass was then increased to 1000 cc. by adding water, and solidified.

At temperatures ranging from 17 to 20° Germination of Greeley County bunt occurred in from 6 to 9 days and sporidial production in 12 days. "White colonies" developed in three more days.

For the same medium acidified with one drop of lactic acid per plate, very little germination occurred in 15 days, at which time the medium began to dry out.

Results at room temperature could not be satisfactorily determined because of the tendency of the heavy medium to dry out quickly.

Carrot Two Per Cent Dextrose. This medium was made by boiling 200 gr. of carrots in 500 cc. of water until the carrots were well cooked up. The extract was then filtered through cotton. Dextrose, and agar for solidifying were added with water to make up to 1000 cc. The medium was autoclaved at 10 lbs. pressure for 15 minutes.

A low percentage of germination of Greeley County bunt occurred on this medium, perhaps less than 2 per cent, when kept at 18 to 20° C. for 15 days. No sporidia were

produced. Promycelia remained short or somewhat branched into club-like appendages.

At room temperature germination occurred in 9 days. No sporidia were produced.

Wheat Extract Agar. This medium was made by soaking 100 gr. of Kanred wheat overnight, boiling one-half hour the following day, and sterilizing and solidifying the filtered broth.

At room temperatures (23 to 25° C.) germination of Greeley County bunt occurred in 4 days, with the production of "white colonies" in 6 days. Sporidia were distorted and often unusually long. This medium is considered good for the starting of bunt cultures.

Acid added to this medium seemed to prevent the germination of chlamydospores.

Soil Extract-Asparagin Agar. This type of medium was prepared by the general method described by Kienholz and Heald (13), except that the concentration of the soil extract was varied and different percentages of asparagin added to each concentration as indicated in Table VII.

Table VII.--Germination tests with chlamydospores of Tilletia laevis, Greeley County bunt, on soil extract agar plus asparagin, March, 1931.

Type of soil	Per cent asparagin	Type of germination, 10 days	
		500 cc. H_2O per 75 gr. soil	1000 cc. H_2O per 75 gr. soil
Loam	0.2*	Few sporidia, more or less regular in shape; per cent of germination: 1 to 10. Promycelia not branched.	Slight germination. No sporidia.
	0.5	Many sporidia formed. Generally regular in shape. Germination 10 to 20 per cent. Promycelia not branched.	Small percentage of germination with a few sporidia produced. Promycelia not branched.
	1.0	Germination less than 5 per cent. Short promycelia. No sporidia formed. Many spores burst spreading granular contents.	No germination.
Silt	0.2	No sporidia formed. Germination around 10 per cent. Promycelia not branched.	No germination.
	0.5	Few sporidia produced. Germination 10 per cent. Promycelia not branched.	Small percentage of germination. One set of sporidia noted in 3 plates. Promycelium not branched.
	1.0	Germination less than 5 per cent. Short promycelia. No sporidia formed.	No germination.

Clay:	0.2	:Germination of a few spores. No sporidia. Short promycelia.	:No germination.
	0.5	:Germination 5 per cent. No sporidia. Short promycelia.	:Few spores germinated.
	1.0	:No germination.	:No germination.

*Four plates for each per cent of asparagin in each soil extract concentration were inoculated with Greeley County smut.

Germination of chlamydospores of bunt on this medium at temperatures from 17 to 20° C. varied for the concentration of soil extract and the amounts of asparagin present. In general, loam and silt soil extract (500 cc. water to 75 gr. soil plus 0.5 per cent asparagin) gave the best production of sporidia, which were more or less regular in shape, often showing distinctly the H-shaped pairs of sporidia. Sporidia were observed to germinate on the loam soil extract agar (500 cc. water to 75 gr. soil plus 0.5 per cent asparagin) in 12 to 15 days.

Only one collection of bunt was tested on this medium because of the lack of incubator space and the limited time available. It is not possible to say whether the other collections of bunt would have reacted similarly. A second test of the Greeley County collection was not conducted to check the results of the first.

In general, it may be said that soil asparagin agar is not so suitable for observations on chlamydospore and sporidial germination as plain agar because the medium is invariably cloudy.

Germination tests at room temperatures showed soil extract agar to be less satisfactory for the production of "white colonies" than plain agar. Chlamydospore germination appeared to be 90 per cent or more, but sporidial development was not profuse.

Cultural Studies

The next step after the determination of the best media for chlamydospore germination was to initiate colony growth and study the cultural characters of those collections of bunt selected as representative of the apparently distinct pathogenic strains.

Before spore plantings were made for this purpose, however, a microscopic examination was made of all bunt collections, 1928 to 1930, inclusive, to determine by spore-wall markings the species of bunt represented. All collections were thought to be Tilletia laevis and were originally marked as such. The writer had previously examined and become familiar with the spore-wall characters of a collection of bunt in the Kansas Station Herbarium known to be Tilletia tritici (Bjerk.) Wint. He then examined under oil immersion several samples of each Kansas collection of bunt. Considerable variation in size and shape of chlamydospores could be noted in the various collections, but echinulations were not observed. All collections of chlamydospores showed the smooth wall character of Tilletia laevis.

An attempt was made, in as far as possible, to start cultures of the various representative collections of bunt

at the same time. Spore plantings were made on plain agar as explained above under Germination Studies. Plates were incubated at room temperatures and transfers made from these to media suitable for growth, when the "white colonies" had developed to the size of a pinhead or larger.

Wheat bunt is an extremely slow grower on artificial media, and consequently it was deemed advisable to grow the cultures in flasks to retain sufficient moisture in the medium and lessen the possibility of contamination. Transfer from the plates was made by lifting a "white colony" with the point of a small scalpel and placing it with a small bit of medium on the medium in a flask. Very infrequent contamination resulted when this process was carried out in the transfer chamber.

Limited incubator space was available so that it was not possible to study the growth characters of the various bunt collections on more than two media at one time. A preliminary test with bunt from Greeley and Meade Counties, Kansas, was performed to determine something of the relative possibilities for strain differentiation in potato 4-per cent sucrose, potato 2-per cent dextrose, and oatmeal 3-per cent dextrose agar. Potato 4-per cent sucrose and

oatmeal 3-per cent dextrose agar were considered the best in differentiating properties. Wherever possible, at least three flask cultures were made of each representative collection of bunt. Brown and Barton County smut were limited to 2 and 1 respectively, because of difficulties encountered in obtaining bacteria-free smut colonies. The flask cultures were incubated at 17 to 20° C. for two months.

Plate I shows the cultural characters of six collections of bunt, representing five pathogenic strains. The cultures were photographed after one month's growth on potato 4-per cent sucrose agar. Tables VIII and IX present summaries of the cultural characters of the six collections of bunt pictured in Plate I. The cultural characters of three pathogenic strains of Kansas bunt grown on potato 4-per cent sucrose and oatmeal 3-per cent dextrose agar are illustrated in Plate II. The cultures were photographed after one month's growth. Tables X and XI give summaries of the cultural characters of three bunt strains represented in the collections from Greeley, Meade, and Lane Counties, grown on oatmeal 3-per cent dextrose agar.

Table VIII.--Summary of cultural studies of Tilletia laevis on potato 4-per cent sucrose agar, at one month after planting. April 13, 1931.

Source of smut	Year collected	Diam. of culture	Color	Consistency	Colony character	Color of medium
		mm.				
Barton County (on Turkey)	1928	19	Pure white	Velvety with cottony margins	Raised 2 mm.; few plaster-like white knobs at the center; margin irregular, more or less definite; no sporidial discharge as indicated by lack of outlying colonies; surface of colony slightly furrowed.	Unchanged.
Meade County	1929	18	White	Same.	Raised 5 mm.; margin irregular but definite; irregular mounds at the center with clear exudate droplets; sporidial discharge as indicated by outlying colonies; surface slightly furrowed.	Slightly browned around colony.
Lane County	1929	17	Dirty white	Cottony	Flat except for a few knobs of variable height up to 2 mm. around the center; margin somewhat regular and definite; no sporidial discharge; few clear exudate droplets on knobs; surface not furrowed.	Reddish-brown.

Johnson County (on Harvest Queen)	1928	15	Bluish-white. Few white cottony knobs.	Velvety	Flat, except for a few knobs at the center; margin irregular and definite; no sporidial discharge; no exudate droplets; surface slightly furrowed.	Slightly browned around colony.
Brown County	1929	16.5	Same.	Velvety	Flat except for knobs of variable height at center; margin irregular and definite; few droplets of exudate on knobs.	Same.
Greeley County	1929	10 X 5	Dirty white with large pure white knobs.	Plastery to cottony	Raised 8 mm.; medium raised beneath the colony; sporidial discharge; margin definite and irregular. No exudate droplets.	Unchanged.

Table IX.--Summary of cultural studies of Tilletia laevis on potato 4-per cent sucrose agar at two months after planting. May 13, 1931.

Source of smut	Year collected	Diam. of culture mm.	Color	Consistency	Colony characters	Color of medium
Barton County (on Turkey)	1928	26	Pure white	Velvety with cottony margins	Raised 4 mm.; few plaster-like white knobs at the center; margin irregular and definite; no sporidial discharge; few exudate droplets; surface of colony slightly furrowed.	Unchanged.
Meade County	1929	25	White	Velvety with cottony margins	Raised 6 mm.; margin irregular and definite; irregular mounds at center; sporidial discharge; few exudate droplets light brown; surface irregular and furrowed.	Slightly browned around colony.
Lane County	1929	21	Dirty white.	Cottony	Flat except for knobs bearing exudate droplets at center; colony nearly circular; margin irregular and definite; surface slightly furrowed.	Deep brown throughout

Johnson County (on Harvest Queen)	1928	23 X 20	Bluish-white, few white cottony knobs	Velvety	Flat except for few scattered knobs; margin irregular and definite; no sporidial discharge; no exudate droplets; surface furrowed, making V sectors	Slightly browned throughout
Brown County	1929	25	Same.	Velvety	Flat except for few scattered knobs; margin irregular and definite; no sporidial discharge; few droplets of exudate on knobs; surface furrowed making V sectors.	Slightly browned throughout
Greeley County	1929	15 x 10	Dirty white with large white knobs.	Plastery to cottony	Raised 10 mm.; medium raised beneath culture; margin very definite and irregular; no exudate droplets; sporidial discharge; circular furrows around colony mound.	Unchanged.

Table X.--Summary of cultural studies of Tilletia laevis on oatmeal 3-per cent dextrose agar at one month after planting.

Source of smut	Year collected	Diam. of culture	Color	Consistency	Colony character	Color of medium
		mm.				
Greeley County	1929	15 X 12	Pure white	Velvety	Raised 5 mm; more or less circular; margin regular and definite; no sporidial discharge; many exudate drops over surface; surface not furrowed.	Slightly purplish or grape-colored around colony.
Lane County	1929	9 X 8	Dirty white	Velvety with cottony margin	Flat; margin slightly irregular but definite; no exudate droplets; slow growing.	Grape-colored around colony.
Meade County	1929	12	Dirty white	Same.	Raised 2 mm.; margin slightly irregular but definite; many exudate droplets.	Unchanged.

Table XI.--Summary of cultural studies of Tilletia laevis on oatmeal 3-per cent dextrose agar at two months after planting.

Source of smut	Year collected	Diam. of culture	Color	Consistency	Colony character	Color of medium
		mm.				
Greeley County	1929	29 X 24	Pure white	Velvety	Raised 6 mm.; circular colony; margin regular and definite; no sporidial discharge; very numerous exudate droplets light orange in color over surface at center.	Slightly grape-colored around colony.
Lane County	1929	15 X 12	Dirty white	Velvety with cottony margin	Flat; margin irregular and definite; no sporidial discharge; no exudate droplets; slow growing.	Same.
Meade County	1929	16	Same.	Same.	Raised 2 mm.; margin irregular and definite; surface irregular and slightly furrowed.	Unchanged.

The descriptions are based upon typical cultures of the various pathogenic strains of bunt. Likewise, the cultures shown by photograph were selected as typical and representative. It was not possible to show more than one culture of each bunt strain.

Substantial differences in cultural characters are manifest among the various pathogenic strains of bunt grown on potato 4-per cent sucrose and oatmeal 3-per cent dextrose agar. Slight differences occurred among the mass spore cultures of some of the individual strains, but, as a rule, duplicate cultures of single pathogenic strains showed all or practically all characters in common. Cultures of Lane and Greeley County bunt were extremely constant in characters on both media.

Incubator space permitted the growth and comparison in cultural characters between two collections of bunt from a single pathogenic strain. Striking similarities exist between the cultural characters of these two collections (Brown County 1929 and Johnson County 1928) when grown on potato 4 per cent sucrose agar. Kienholz and Heald (13) noted changes in cultural characters in successive transfers of several of their bunt cultures on artificial media. No statement can be made as to whether the similarities in the two cultures mentioned above will or

will not persist through subsequent transfers on the same medium. Time and incubator space have not permitted the study of subsequent transfers of any of the bunt cultures, so that it is not possible to say whether differences in the initial mass-spore cultures will maintain themselves. Additional s tudy will be necessary to determine this point.

SUMMARY

1. Increasingly severe outbreaks of wheat bunt in Kansas in recent years tend to indicate the occurrence of more virulent strains of the bunt organism within the state.

2. In the wheat growing seasons, 1928-29 to 1930-31, tests were conducted in the nursery of the Department of Botany and Plant Pathology at Manhattan, Kansas, to determine the reaction of 16 selected varieties of winter wheat to 36 collections of bunt from various wheat growing sections in Kansas.

3. All collections of Kansas bunt were found on microscopic examination to belong to the species Tilletia laevis Kühn.

4. Distinct differences in virulence among the bunt collections were manifested on the varieties Turkey

C.I. 1558-A, Martin C.I. 4463, Hussar C.I. 4843, White Odessa C.I. 4655, and Banner Berkeley C.I. 7362.

5. The collections of bunt have been grouped according to their types of virulence on the varieties listed in (4), into six pathogenic groups. These groups may properly be designated as physiologic forms.

6. On the basis of the grouping in (5) a dichotomous key has been prepared for the identification of the six physiologic forms of bunt in Kansas.

7. On the basis of the bunt collections tested, a map has been prepared showing the distribution of the physiologic forms of bunt in Kansas. Form I or the common form of bunt is the most widely distributed.

8. Back inoculations with their own smut, on resistant varieties of wheat showing small percentages of bunt in the 1930 differential tests, resulted in increased infection in 10 cases out of 32. Certain large increases in infection tend to indicate the segregation of strains of bunt virulent on certain varieties.

9. The wheat varieties Oro C.I. 8220, Turkey X Bearded Minnesota #48 C.I. 8243, and Minturki X Bel Buf. C.I. 8033 show resistance to all forms of Kansas bunt. Martin C.I. 4463, Ridit C.I. 6703, Hussar C.I. 4843, White Odessa C.I. 4655, Banner Berkeley C.I. 7362, Regal

C.I. 7364, and Cooperatorka C.I. 8861 show resistance to most forms of bunt. These varieties may be used as sources of bunt resistant parents in crosses with hard red winter wheats for Kansas use.

10. Laboratory studies have shown that plain agar, soil extract agar, and wheat (grain) extract agar are satisfactory for the germination of chlamydospores of Tilletia laevis and for the initiation of colony growth.

11. Potato 4-per cent sucrose and oatmeal 3-per cent dextrose agar were found to be the best media for showing cultural differences among pathogenic strains of bunt. Consistent and substantial differences in cultural characteristics were shown to exist among representative bunt collections from five distinct pathogenic groups.

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Plate I. Thirty-day-old mass-spore cultures of six representative collections of bunt from five distinct pathogenic groups on potato 4-per cent sucrose agar.

1. Brown County bunt, 1929. Group II.
2. Johnson County bunt, 1928. Group II.
3. Greeley County bunt, 1929. Group I.
4. Lane County bunt, 1929. Group IV.
5. Meade County bunt, 1929. Group III.
6. Barton County bunt, 1928. Group V.

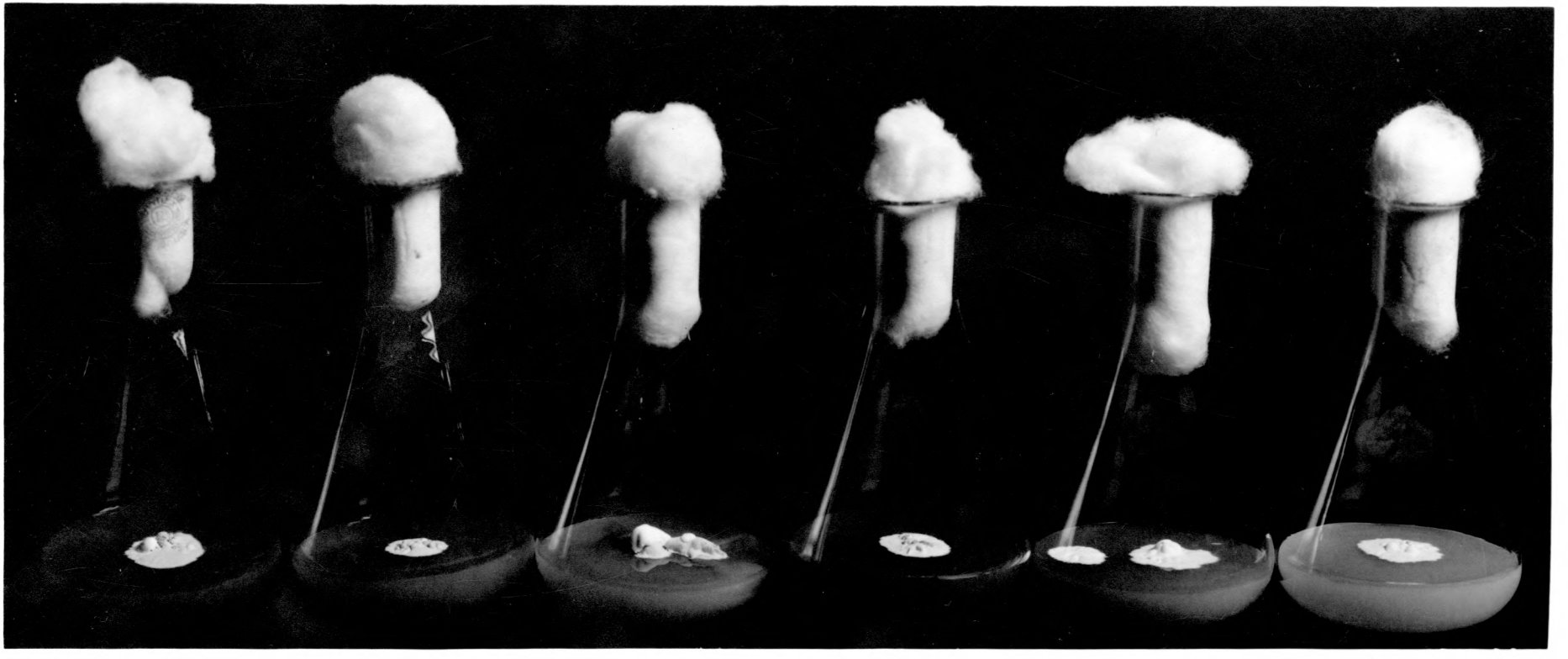

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Plate II. Thirty-day-old mass-spore cultures of three representative collections of bunt from three distinct pathogenic strains on oatmeal 3-per cent dextrose and potato 4-per cent sucrose agar.

1. Greeley County bunt, 1929. Group I. Oatmeal 3-per cent dextrose agar.

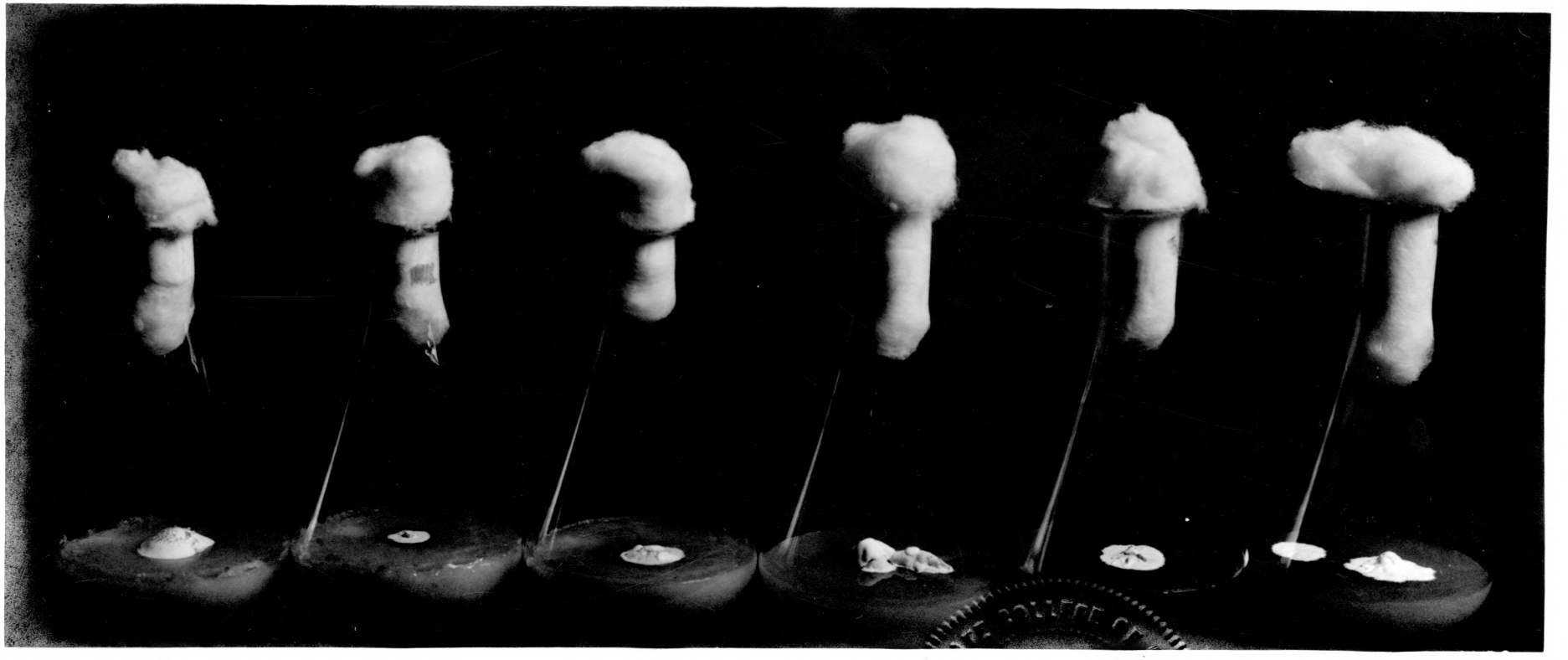
2. Lane County bunt, 1929. Group IV. Oatmeal 3-per cent dextrose agar.

3. Meade County bunt, 1929. Group III. Oatmeal 3-per cent dextrose agar.

4. Greeley County bunt, 1929. Group I. Potato 4-per cent sucrose agar.

5. Lane County bunt, 1929. Group IV. Potato 4-per cent sucrose agar.

6. Meade County bunt, 1929. Group III. Potato 4-per cent sucrose agar.



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