RESEARCH BULLETIN 560 SEPTEMBER, 1954

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

J. H. LONGWELL, *Director*

RESISTANCE OF WINTER BARLEY TO *USTILAGO NUDA* (Jens.) Rostr.

C. K. CLONINGER AND J. M. POEHLMAN

(Publication authorized August 31, 1954)

COLUMBIA, MISSOURI

CONTENTS

RESISTANCE OF WINTER BARLEY TO *USTILAGO NUDA* (Jens.) Rostr.

C. K. CLONINGER AND J. M. POEHLMAN[1]

INTRODUCTION

Loose smut, *Ustilago nuda* (Jens.) Rostr., is one of the major diseases of winter barley in Missouri. The smutted plants compete with the normal healthy plants for moisture and nutrients but fail to produce grain. The disease causes a reduction in yield approximately equivalent to the percentage of spikes of loose smut present (7, 38, 47).[2] The true or "deep-borne" loose smut, develops from floral infection and can be controlled in susceptible varieties only by the difficult-to-use hot water or water soaking (52, 15) methods of seed treatment. The use of resistant varieties, therefore, becomes the most practical means for the control of this disease.

At the time this study was initiated, the leading winter barley varieties in Missouri were Reno, Michigan Winter, Kentucky 1, and Missouri Early Beardless. All of these varieties except Missouri Early Beardless were highly susceptible to loose smut. While Missouri Early Beardless seldom contained as high a percentage of smut infection as other varieties, it was not considered resistant. However, it later was learned that smut resistant strains could be selected from Missouri Early Beardless as well as from hybrid populations in which Missouri Early Beardless selections had been used as parents (26, 27). The question then arose as to whether there might be physiologic forms of *U. nuda* in Missouri and other states in the winter barley area which might attack Missouri Early Beardless selections or the hybrids which had derived their resistance from this parentage. This study was made to clarify that point. A series of varieties and experimental strains of winter barley were inoculated with a large number of *U. nuda* specimens obtained in Missouri and other states in the winter barley area. Results of the inoculations indicated the presence of physiologic specialization. Prior to this study, little was known regarding the possible existence of physiologic forms of *U. nuda* in the winter barley region.

LITERATURE CITED

Sources of Resistance to *Ustilago nuda* (Jens.) Rostr.—Shands and Shaller (39) reported an extensive study on the reaction of spring barley varieties to inoculation with *U. nuda.* In testing 300 varieties they found 4

[1]Acknowledgement is made to Dr. M. E. Michaelson for helpful suggestions during the preparation of this manuscript.

[2]References to literature cited in the Bibliography.

to have 100 percent infection, 36 to have less than 5 percent infection, while the remaining varieties were rather evenly distributed among the infection classes 4-15 percent, 16-30 percent, 31-60 percent, and 61-100 percent. Of the 36 varieties with less than 5 percent loose-smut infection, 9 varieties had no infection.

Poehlman (26) in 1947 gave a preliminary report on the sources of varietal resistance in winter barley to *U. nuda.* Variety reactions were obtained by artificial inoculations with a composite of smut collected from varieties growing in the breeding nursery at Columbia. The varieties were separated into five groups on the basis of origin and lemma appendage. Varieties averaging 5 percent infection or less were considered resistant; varieties averaging 5.1 to 17 percent infection were considered moderately susceptible; and varieties averaging above 17 percent infection were considered susceptible. The five groups into which the varieties were divided and the number of varieties classified as resistant or only moderately susceptible in each were as follows.

(1) Rough-awned Tennessee winter types, or those of closely related origin—6.
(2) Smooth-awned winter barley varieties and selections—0.
(3) Winter barleys of foreign origin—1.
(4) Winter barleys selected from composite crosses—3.
(5) Hooded winter barley varieties and selections—17.

Although Kentucky 6 of the Tennessee Winter group was classified as resistant, attention was called to field infection which suggested the possibility of physiologic specialization. As further evidence of physiologic specialization, the variety Davidson, from a composite cross group, also showed a varied reaction. Many resistant lines were found among the hooded types. Most of these are of similar parentage having originated, apparently, from a cross of Tennessee Winter and a spring beardless type (19). Many hooded selections from the variety Missouri Early Beardless, which originated as a mass selection from a barley believed to be of Tennessee Beardless origin, were resistant.

Poehlman (28) found also that from a group of 165 barleys of foreign origin, 34 were resistant to *U. nuda.* Of the 34 resistant varieties, 11 were from Korea, 6 from Russia, 5 from China, 4 from Caucasus, 4 from Transcaucasus, and 1 each from Holland, Persia, and Afghanistan. The need for additional testing of the varieties resistant to varied collections of *U. nuda* to ascertain the quality of their resistance was stressed. Varieties possessing resistance to the largest number of smut collections would be of greatest value as parent material in a breeding program. Many of the strains resistant to *U. nuda* also were resistant to *U. nigra* Tapke, to *U. hordei* (Pers) Lagerh, or to both. The varieties from foreign origin and from the hooded

selections appeared to offer the best source of germ-plasm in winter barley for resistance to *U. nuda.*

Physiologic Specialization of Pathogenic Fungi—Tervet (48) observed that in breeding for resistance to disease it is necessary to know the response of potentially useful breeding varieties to not only one collection but also to a representative sample of collections of the pathogen in order to determine if physiologic specialization of the organism exists. Physiologic races, determined by differences in pathogenicity on different host varieties, have been demonstrated for many of the pathogenic fungi. Some workers (32, 41) have chosen to distinguish between physiologic forms on the basis of cultural characters, physio-chemical reactions, and morphology in addition to pathogenicity. The early classical work on physiologic specialization was done in the rusts and powdery mildew of cereals but many examples also have been observed in the smut fungi (1, 2, 5, 8, 10, 11, 12, 23, 24, 29, 30, 31, 32, 33, 34, 36, 43, 45, 46, and others).

Physiologic Specialization in *Ustilago nuda*—Nahmmacher (21) in Germany in 1932 reported two distinct physiologic races of *U. nuda* among 45 collections from different sources. Vanderwalle (53) in the same year described an early form and a late form of *U. nuda.* Thren (50) in 1941 demonstrated winter and summer races of *U. nuda.* Tapke obtained evidence of several physiologic races of *U. nuda* based on their reaction on five varieties of spring barley.[3] Shands (40) reported a new race of *U. nuda* which infected Trebi plants, but which did not infect the variety Anoidium.

Artificial Inoculation Techniques—The purpose of any inoculating technique is to place the loose smut chlamydospores on the ovaries of the florets during flowering time. Many techniques have been used but, generally speaking, all may be classified into two groups: (a) The introduction of dry chlamydospores into the florets (3, 9, 21, 24, 37, 38, 39, 42, 50), and (b) the introduction of a chlamydospore-suspension into the florets (1, 3, 20, 25, 35, 55). The variations in the manner of introduction of the spores into the florets constitute the differences in methods. None of the methods give extremely high infection percentages consistently. Comprehensive reviews of the literature on smut inoculation have been made by Shands and Schaller (39), Cloninger (6), and Johnston (14).

Shands and Schaller compared the vaccuum method of inoculation, described by Moore (20); the use of forceps; clipping the upper part of the floret and dusting with dry spores; and a method where individual flowers were inoculated by use of a hypodermic needle attached to a small rubber bulb containing dry chlamydospores. The last procedure, known as the "needle" method, gave the highest infection percentage of all. Ross *et al.* (35) compared several methods of inoculation including a dry spore brush

[3]Personal correspondence with Dr. V. F. Tapke, formerly with Section Cereal Crops and Diseases, U. S. Department of Agriculture, Beltsville, Md.

method and the syringe "spore-suspension" method. They obtained significantly higher infections from the "spore-suspension method."

Poehlman (25) described a "bulb-suspension" method using a chlamydospore-suspension which was injected into individual flowers by means of a hypodermic needle and a rubber bulb. Bever (1, 2), in his studies of *Ustilago tritici,* injected a 1 percent malt, chlamydospore-suspension into each floret with a small, medical hypodermic syringe. Tapke[3] in his studies with *U. nuda* used a sterilized water chlamydospore-suspension injected into each floret by a medical hypodermic syringe. Inoculations in all cases were made at or immediately following anthesis. None of the methods of inoculation gave consistently high infections, and considerable variability in percentage of smutted heads or plants was noted in the results of many workers.

MATERIALS AND METHODS

Winter Barley Varieties Inoculated—A total of 58 varieties of winter barley and 34 collections of *U. nuda* were used in this study. The varieties, the "C. I."[4] number, the source or origin of each, and the number of years inoculated with each of 13 representative collections of *U. nuda* are listed in Table 1. Only 21 barley varieties were selected to initiate the study in 1947. Even though it was known that some of these varieties were highly susceptible to *U. nuda,* they were included to ascertain whether they were susceptible to collections of inoculum obtained from widely separated sources. The reaction of other varieties was unknown. As the investigation progressed it became evident that many of the varieties were susceptible to all of the collections of *U. nuda* being used. Most of the susceptible varieties were discontinued after the first year. Other varieties with unknown reactions or which showed some degree of resistance were added. Many of the hybrid selections from the Missouri Agricultural Experiment Station breeding nursery and introductions obtained from the United States Department of Agriculture were inoculated with selected collections only in the latter phases of the study.

Collections of *Ustilago nuda* Used as Inoculum—During the 1946 and 1947 seasons, 229 collections of loose smut were obtained. Thirty of these original collections, after positive identification of the species on the basis of the type of spore germination (44), were used in this study. Of these, eleven came from widely separated regions in Missouri, six from Texas, four from North Carolina, three from Arkansas, and one each from Mississippi, West Virginia, New Jersey, Illinois, Nebraska, and Kentucky. In 1950, four additional collections, two from Missouri and two from Kentucky, were added to the group. The collection number, the variety from

[4]Accession number of the Section of Cereal Crops and Diseases, U. S. D. A.

TABLE 1 -- THE NUMBER OF YEARS WHICH EACH VARIETY WAS INOCULATED WITH EACH OF THIRTEEN REPRESENTATIVE COLLECTIONS OF USTILAGO NUDA*

Variety or Selection	Source or Origin	C. I.+ Number	Number of Years Inoculated with Collection Number: 1	2	4	5	9	10	11	14	15	17	25	29	35
I. Rough-Awned Tennessee Winter Type:															
Reno		6561	5	7	5	5	5	4	6	4	4	3	4	3	3
Michigan Winter		2036	1	1	1	1	1	1	-	-	-	-	-	-	-
Purdue 21		4581	1	1	1	1	1	1	-	-	-	-	-	-	-
Kentucky 6		4678	3	4	3	3	3	3	1	1	1	1	2	3	-
Ward		6007	2	2	1	1	1	1	1	1	1	-	-	-	-
Kentucky 1		6050	3	4	3	3	4	2	2	2	2	1	-	1	-
II. Smooth-Awned Varieties of Tennessee Winter Type Origin:															
Smooth-awned 86		6668	1	1	1	2	1	1	1	1	1	-	-	-	-
III. Introduced Varieties of Commercial Importance:															
Alaska		534	1	1	1	1	1	1	-	-	-	-	-	-	-
Sunrise		6272	1	1	1	1	1	1	-	-	-	-	-	-	-
Poland		6280	3	4	3	3	3	3	1	1	1	1	2	3	-
Wong		6728	1	1	1	1	1	1	1	1	1	-	-	-	-
IV. Selections from Composite Crosses:															
Davidson		6373	3	3	2	2	2	4	1	2	1	1	2	3	-
Nassau		7022	2	3	2	2	3	3	2	2	2	2	1	1	-
Harbine		7524	-	1	-	-	-	-	1	-	-	-	1	-	1
Kenbar		7574	-	1	-	-	-	-	1	-	-	-	1	-	1
V. Hooded Varieties of Tennessee Beardless or Similar Origin:															
Hooded 16		6574	3	5	2	3	3	3	3	2	1	1	2	1	3
North Carolina Hooded 26		7026	5	7	4	5	5	4	6	3	4	3	3	2	3
Tucker		7039	4	4	3	3	4	2	1	1	1	1	1	3	-
B351‡	M.E.B. Sel.		4	6	4	4	4	3	4	3	3	3	4	3	2
B355	M.E.B. Sel.		1	2	2	1	1	1	-	-	-	-	-	1	-
B405	M.E.B. Sel.		3	5	3	3	3	3	2	2	2	2	3	1	2
B411	M.E.B. Sel.		1	3	1	1	2	2	1	-	-	1	1	1	1
B495	M.E.B. Sel.		-	1	-	-	-	-	1	-	-	-	1	-	1
B502	M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B538	M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B467	Kinroku Sel.		3	5	3	3	3	3	5	3	3	3	3	2	3

TABLE 1 -- CONTINUED

Variety or Selection	Source or Origin	C. I.+ Number	Number of Years Inoculated with Collection Number: 1	2	4	5	9	10	11	14	15	17	25	29	35
VI. Selections of Hybrid Origin with Missouri Early Beardless Parentage:															
Mo. B-400	Ky. 5 x M.E.B. Sel.	7568	2	5	1	1	1	1	3	1	1	1	3	1	2
B575	Admire x M.E.B. Sel.	9168	1	4	1	1	2	2	2	-	-	1	2	-	2
B580	Admire x M.E.B. Sel.	7567	3	5	3	3	3	3	5	3	3	3	4	2	3
B588	Admire x M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B631	Admire x M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B635	Admire x M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B698	Admire x M.E.B. Sel.		1	2	1	1	2	2	-	-	-	1	-	-	-
B699	Admire x M.E.B. Sel.	8064	1	4	1	1	2	2	2	-	-	1	2	-	2
B637	Mich. Winter x M.E.B. Sel.	7571	-	2	-	-	-	-	2	-	-	-	2	-	1
B640	Ward x M.E.B. Sel.	7572	1	4	1	1	2	2	2	-	-	1	2	-	2
B696	Ky. 2 x M.E.B. Sel.		2	4	2	2	2	2	4	2	2	2	4	2	2
B703	B289 x M.E.B. Sel.		1	4	1	1	2	2	2	-	-	1	1	-	2
B705	Ky. 5 x M.E.B. Sel.	9169	-	1	-	-	-	-	1	-	-	-	1	-	1
B757	Admire x Dorsett		-	1	-	-	-	-	1	-	-	-	1	-	1
B767	Admire x Dorsett		-	1	-	-	-	-	1	-	-	-	1	-	1
VII. Selections from U.S.D.A. World Collection:															
Han River	China	206	1	4	1	3	2	2	-	-	-	1	-	-	-
Omar	Hybrid	898	1	4	1	3	2	2	-	-	-	1	-	-	-
Clancy	Russia	1002	1	4	1	3	2	2	-	-	-	1	-	-	-
Abundio	Persia	1412	-	3	-	2	1	1	-	-	-	1	-	-	-
	Caucasus	3358	1	4	1	3	2	2	-	-	-	1	-	-	-
	Russia	4959	1	4	1	3	2	1	-	-	-	-	-	-	-
	Russia	4966	0	2	1	1	1	1	1	-	-	-	1	-	1
	China	5087	1	2	1	1	2	2	-	-	-	1	-	-	-
	China	5089	-	1	-	-	-	-	1	-	-	-	1	-	1
	China	5091	1	4	1	3	2	2	-	-	-	1	-	-	-
Omugi	Korea	5144	-	1	-	-	-	-	1	-	-	-	1	-	1
Hokudu	Korea	5176	1	4	1	3	2	2	-	-	-	1	-	-	-
Kada	Korea	5180	1	1	1	1	1	1	-	-	-	1	-	-	-
Saru	Korea	5185	1	2	1	1	2	2	-	-	-	1	-	-	-
Dohadak	Korea	5187	4	6	3	4	3	3	4	3	3	3	3	2	3
Denrai	Korea	5225	-	3	-	2	1	1	-	-	-	-	-	-	-
Dobaku	Korea	5238	1	4	1	3	2	2	-	-	-	1	-	-	-

*Infection percentage in Tables 3 to 7 are an average of the number of years which each variety was inoculated.
+C. I. number refers to accession number of the Cereal Crops Section, United States Department of Agriculture.
‡B number refers to accession number of the Missouri Agricultural Experiment Station.

which collected, and the geographical origin of the 34 collections are listed in Table 2.

Method of Growing Barley Varieties—The original inoculations were made in the barley nursery plots; however, the presence of smut from natural infection made this method unsatisfactory. Therefore, hot-water treated seed of each variety was planted in a "smut-free" nursery, at least 100 yards from all other barley. Plantings were made on two separate dates in order to lengthen the period favorable for inoculation.

It was believed that impurities in the seed might contribute to variations in infection percentages within varieties inoculated with the same collection in different years. To eliminate this possible source of error, pure-line seed increases within each variety were made after the first year. The seed selected for pure-line increases was obtained from individual head-rows

TABLE 2 -- SOURCES OF COLLECTIONS OF USTILAGO NUDA USED AS INOCULUM

Collection Number	Variety from which collected	Where collected
1	Kentucky 1	Washington County, Mississippi
2	Reno	Columbia, Missouri
3	Sunrise	Fayetteville, Arkansas
4	Reno	Denton, Texas
5	Smooth Awn 88	Raleigh, North Carolina
6	Reno	Denton, Texas
7	Mo. Early Beardless	McCredie, Missouri
8	Mo. Early Beardless	Denton, Texas
9	Davidson	Raleigh, North Carolina
10	Tucker	Morgantown, West Virginia
11	B405, M.E.B. Sel.	Columbia, Missouri
12	Nassau	New Brunswick, New Jersey
13	Reno	Perryville, Missouri
14	Reno and Mo. Early Beardless	Columbia, Missouri
15	Mo. Early Beardless	Mt. Vernon, Missouri
16	Michigan Winter	Scott County, Missouri
17	Davidson	Statesville, North Carolina
18	Sunrise	Greensboro, North Carolina
19	Bearded barley	Higginsville, Missouri
20	Reno	Lamar, Missouri
21	Tucker	Denton, Texas
22	Smooth Awn 86	Denton, Texas
23	Purdue 21	Urbana, Illinois
24	Ward	Lincoln, Nebraska
25	B580, Admire x M.E.B. Sel.	Columbia, Missouri
26	Mo. B-400	Pierce City, Missouri
27	Nassau	Fayette County, Kentucky
28	Alaska	Fayetteville, Arkansas
29	Ward	Fayetteville, Arkansas
30	Ward	Denton, Texas
31	Reno	Columbia, Missouri
32	C. I. 2485	Lexington, Kentucky
34	C. I. 5009	Lexington, Kentucky
35	B467, Kinroku Selection	Columbia, Missouri

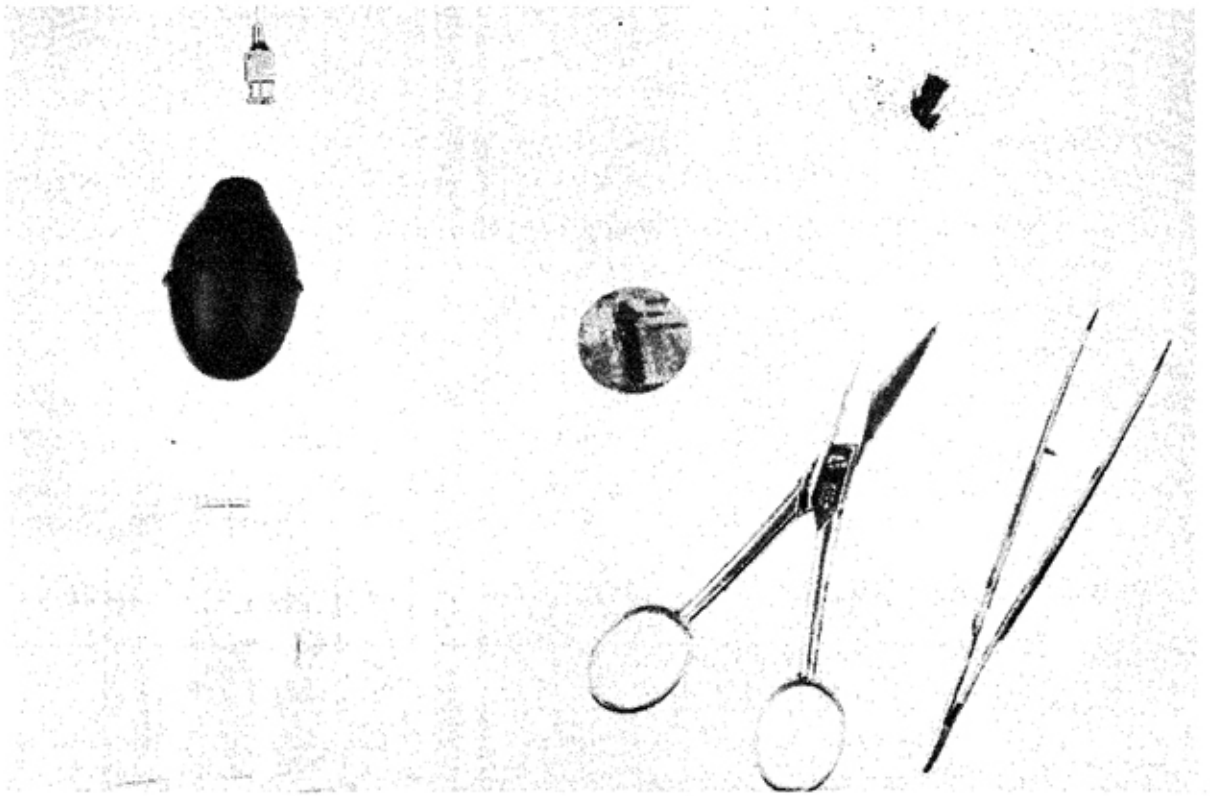

Figure 1. Equipment used in artificial inoculations of barley with *Ustilago nuda* (Jens.) Rostr. On the small square of cheesecloth is shown a small segment of a smutted head to be used in the preparation of a chlamydospore-suspension.

which had been previously inoculated with *U. nuda*. Four head-rows of each variety were harvested and bulked, and the seed was hot-water treated to insure smut-free plants for future inoculations.

Inoculation Procedure—The inoculations reported here were made by the method described by Poehlman (25). The equipment used in making the inoculations consisted of a 1-inch, 25-gauge hypodermic needle inserted into a 10 ml. rubber bulb (Figure 1). The rubber bulb was filled by suction through the needle, with a suspension of chlamydospores (Figure 2d). The suspension was prepared in the laboratory by squeezing the chlamydospores from a section of an individual head through a small square of cheesecloth into a 50 ml. beaker filled with a 2 percent solution of dextrose in distilled water (Figure 2a and b). The chlamydospore suspension was stored in 50 ml. test tubes in the refrigerator at 1° to 2°C. for periods not exceeding 48 to 72 hours (Figure 2c). Different inoculation equipment was used for each collection of *U. nuda* (Figure 2c and d). Before making each spore suspension, the forceps and scissors were sterilized in 70 percent ethyl alcohol.

Four heads of each variety were inoculated by injecting a small quantity of the spore-suspension into each floret of the barley spike one or two days following anthesis (Figure 3). Each inoculated head was harvested

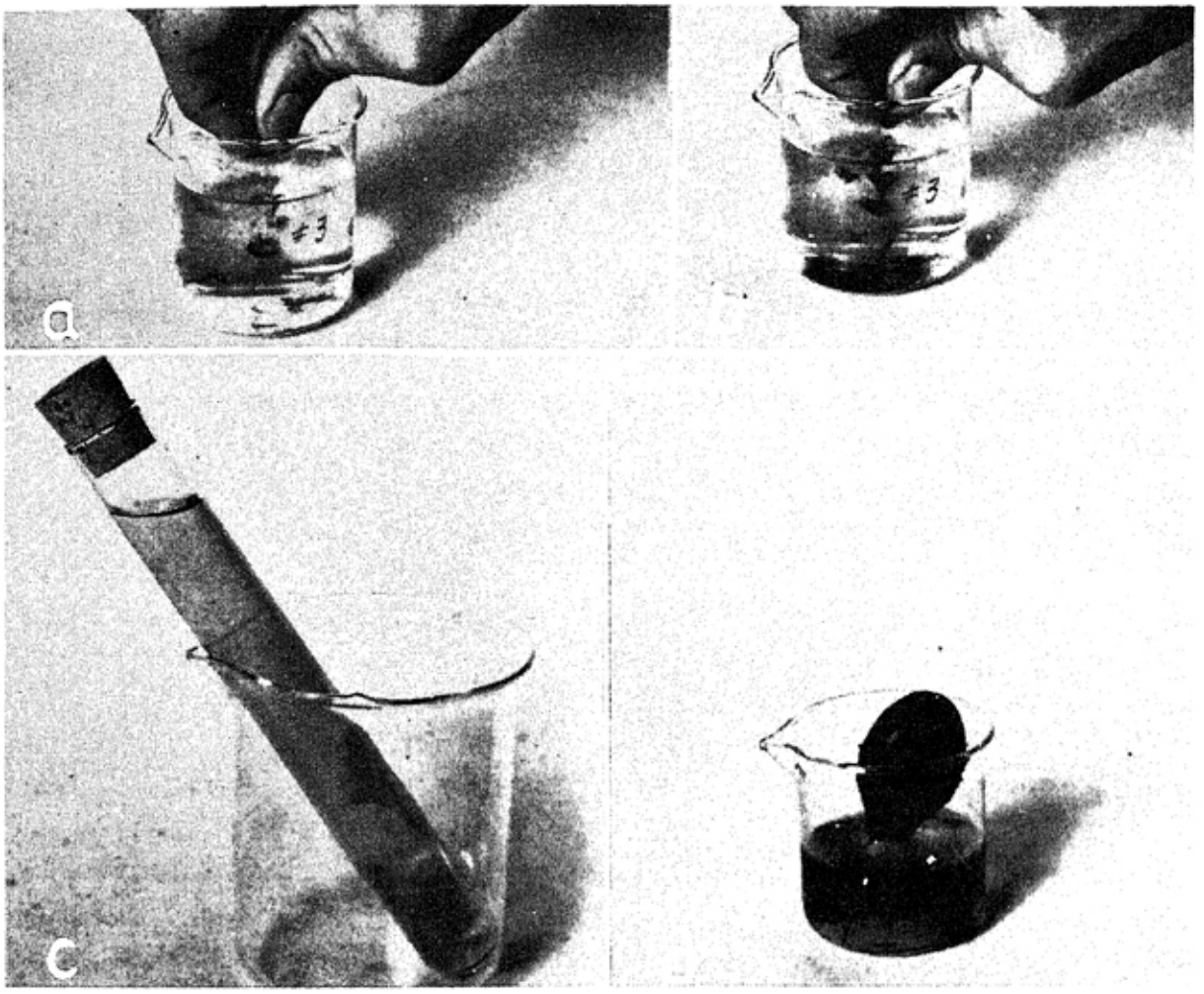

Figure 3. A small quantity of the *U. nuda* chlamydospore-suspension is placed in each floret on the barley spike. The awns are clipped. After inoculation each spike is tagged according to the smut collection and barley variety used.

Figure 2. (a and b) Preparation of a chlamydospore-suspension of *Ustilago nuda* (Jens.) Rostr. by squeezing the spores through the small squares of cheesecloth into a 2 percent dextrose solution, (c) the chlamydospore-suspension ready for storage in the refrigerator, and (d) filling the rubber bulb with the suspension by suction through the needle.

and threshed individually. The seed was treated with New Improved Ceresan diluted in talc before planting. The seed from each of the four inoculated heads was planted in a 1-foot row; a heavy seeding rate was used as a means of reducing winter injury from heaving. Counts of the smutted and the non-smutted heads were made the following spring in each of the four 1-foot rows. The percentage of smutted heads was determined, and the results are given as the average percentage for the four rows.

RESULTS

In this study, each of the 58 varieties and strains of winter barley was inoculated with four or more of 34 collections of *U. nuda* obtained from ten states. At the outset, no information was available regarding either (a) differences in the pathogenicity of the collection being used, or (b) differences in the reactions of varieties that might make them desirable as differentials. The varieties were chosen for inoculations on the basis of resistance as demonstrated by previous inoculations with a single smut collection, provided the variety offered possible usefulness in the barley breeding program at the Missouri Agricultural Experiment Station. The collections of smut used were selected from a wide range of winter barley varieties, and from a broad geographical area. Each variety was inoculated with as many collections as possible each year. It soon was evident that some of the varieties differed in their reaction to the different collections of *U. nuda,* indicating a difference in the pathogenicity of the smut collections being used.

Selection of Differentials—After a study of the infection data obtained, six varieties, North Carolina Hooded 26, B351, B405, B580, Dohadak, and Reno were selected as the varieties which would differentiate best the collections according to differences in pathogenicity.

Three of the varieties chosen as differentials, North Carolina Hooded 26, B351 and B405, are hooded varieties. B351 and B405 are selections from the variety Missouri Early Beardless. B580 is a hybrid selection from the cross Admire x Missouri Early Beardless and derives its smut resistance from the Missouri Early Beardless parentage. Dohadak is an introduction from Korea. Reno was included as a susceptible check variety, since it proved susceptible to every collection with which it was inoculated.

In the analysis of the data, varieties with 10 percent smut infection or less were considered resistant and varieties with 11 percent infection or above were considered susceptible. The 10 percent infection level for dividing resistant and susceptible varieties has some precedence; it was used by Bever (1, 2) in a study of the physiologic specialization of the loose smut organism in wheat. It is recognized that the 10 percent infection level is an arbitrary value and that in the course of many inoculations the percentage infection of different collections may fluctuate above and below this value.

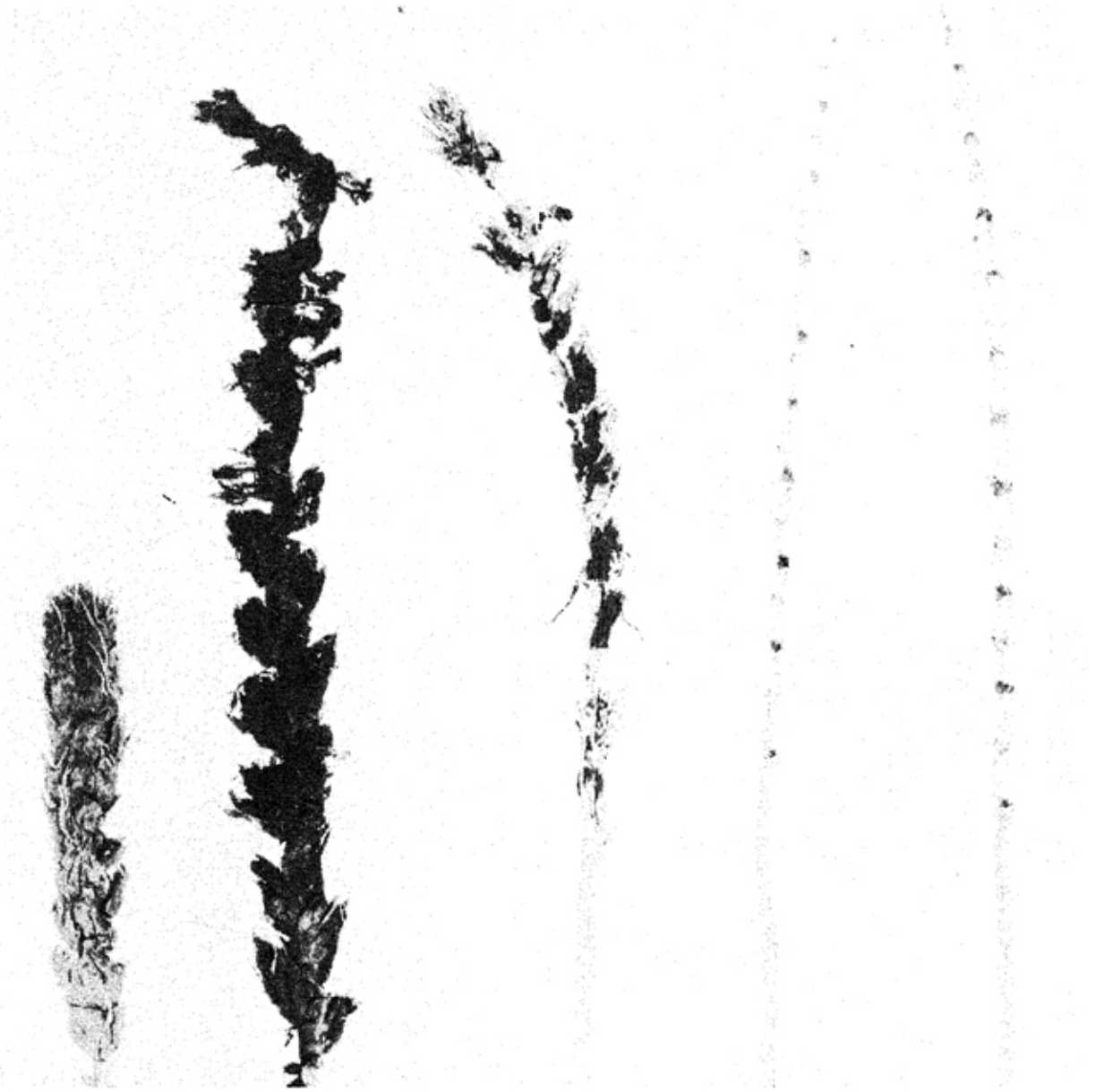

Figure 4. Smutted heads in barley, caused by loose smut, *U. nuda*, (Jens.) Rostr.

Grouping of Collections With Similar Reactions—Having selected the differentials, it was possible to group the collections of *U. nuda* on the basis of similar reaction to the six differential varieties. The 34 collections were divided into 13 groups, with all of the collections in each group showing similar pathogenicity. The grouping of the collections according to the reaction of the six differential varieties of winter barley, is given in Table 3. Varieties with 10 percent infection or less were classified as resistant and those with 11 percent infection or more as susceptible. Two exceptions were made in grouping the collections. B351 was classified as resistant to collection 5, and the collection was grouped with collections 22, 23 and 24 in Group IV, even though it had 11 percent infection. Since the infection percentage exceeded the dividing line by only 1 percent, and since B351

TABLE 3 -- COLLECTION OF USTILAGO NUDA GROUPED ACCORDING TO THEIR PATHOGENICITY AS MEASURED BY THE REACTION OF SIX DIFFERENTIAL WINTER BARLEY VARIETIES

Collection number	Percent Infection in:						Reaction**					
	N. C. Hooded 26	B351	B405	B580	Dohadak	Reno	N. C. Hooded 26	B351	B405	B580	Dohadak	Reno
Group I												
1	1	19	12	21	18	55	R	S	S	S	S	S
27	1	11	33	15	12	41	R	S	S	S	S	S
Group II												
2	1	7	4	14	5	63	R	R	R	S	R	S
3	4	6	3	12	6	66	R	R	R	S	R	S
13	0	0	0	15	3	49	R	R	R	S	R	S
26	0*	4	5	20	3	55	R	R	R	S	R	S
Group III												
4	5	2	9	6	5	70	R	R	R	R	R	S
8	0	1	3	4	2	36	R	R	R	R	R	S
12	0	1	10	6	10	49	R	R	R	R	R	S
16	4	2	4	7	0	46	R	R	R	R	R	S
19	1	3	9	5	0	50	R	R	R	R	R	S
31	0	7	5*	1*	0*	73*	R	R	R	R	R	S
Group IV												
5	6	11	16	8	6	47	R	R?	S	R	R	S
22	1	2	12	5	0	58	R	R	S	R	R	S
23	0	5	16	10	0	44	R	R	S	R	R	S
24	3	1	21	8	0	37	R	R	S	R	R	S
Group V												
9	1	6	49	48	20	63	R	R	S	S	S	S
21	0	7	21	24	28	59	R	R	S	S	S	S
30	5	7*	24*	14	24	53	R	R	S	S	S	S
32	0*	0*	27*	1*	63*	68*	R	R	S	?	S	S
Group VI												
6	4	5	16	13	6	53	R	R	S	S	R	S
7	1	8	11	11	7	55	R	R	S	S	R	S
11	1	4	45	60	4	46	R	R	S	S	R	S
18	1	0	17	15	0	48	R	R	S	S	R	S
20	6	7	13	22	0	46	R	R	S	S	R	S
28	0	2	82*	41	3	54	R	R	S	S	R	S

TABLE 3 -- CONTINUED

Collection number	Percent Infection in:						Reaction**					
	N. C. Hooded 26	B351	B405	B580	Dohadak	Reno	N. C. Hooded 26	B351	B405	B580	Dohadak	Reno
Group VII												
10	1	19	4	9	35	44	R	S	R	R	S	S
Group VIII												
14	13	5	16	13	49	48	S	R	S	S	S	S
Group IX												
15	9	2	1	2	17	24	R	R	R	R	S	S
34	7*	0*	8*	2*	67*	42*	R	R	R	R	S	S
Group X												
17	15	5	56	69	0	62	S	R	S	S	R	S
Group XI												
25	14	1	1	16	29	41	S	R	R	S	S	S
Group XII												
29	2	14	5*	18	0	59	R	S	R	S	R	S
Group XIII												
35	34	17	6	26	40	53	S	S	R	S	S	S

*1 year's data only.
** R = Resistant; S = Susceptible

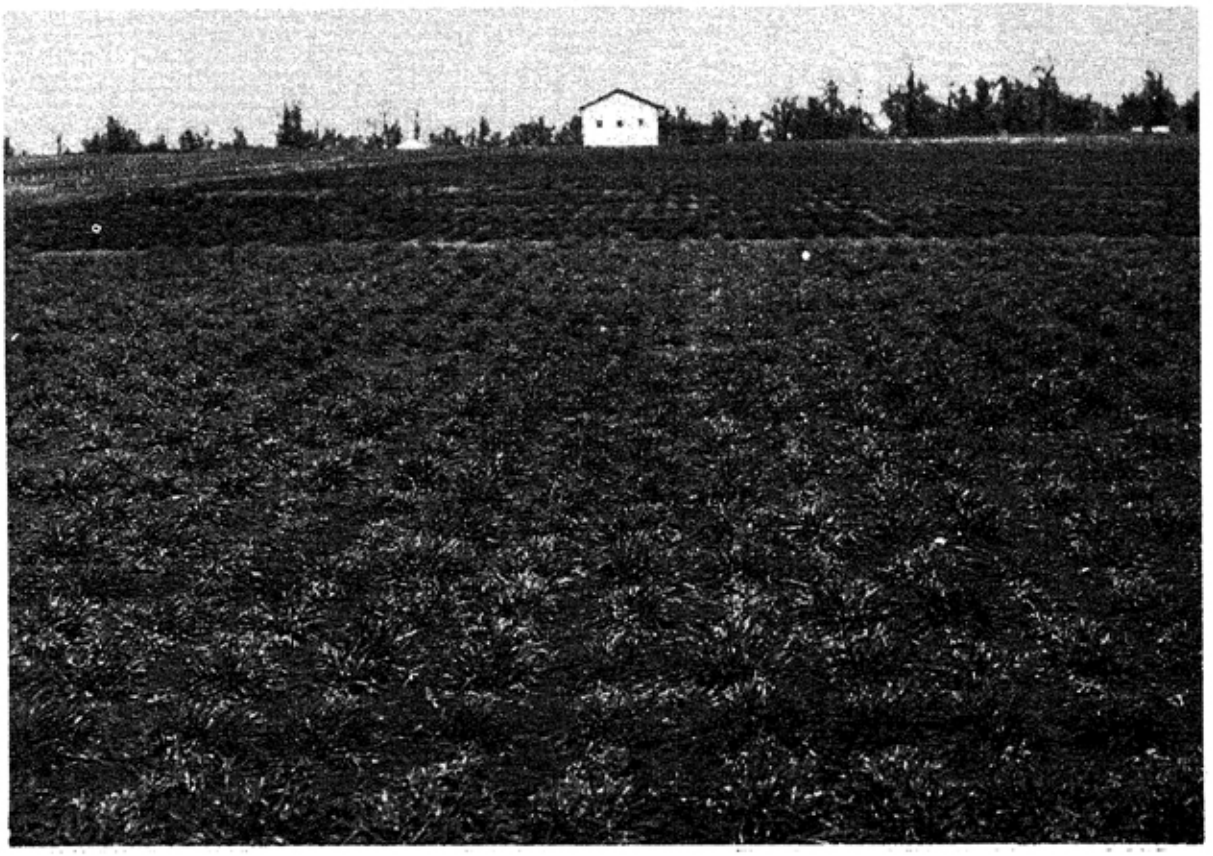

Figure 5. The winter barley loose smut nursery after growth started in the early spring. Note the growth in clumps resulting from planting the seed in 1-foot rows.

was resistant in three of the four years that it was inoculated with collection 5, it did not seem practical to establish a separate group for this collection. Collection 32 produced only 1 percent infection in B580. Since only one year's results were available, this collection was tentatively placed in Group V with collections 9, 21 and 30, to which B580 was susceptible, rather than establish a new group for the single collection.

Reaction of Differential Varieties—The reactions of the six differential varieties to a representative collection from each of the 13 groups of collections of *U. nuda* are presented in Table 4. North Carolina Hooded 26 was resistant to nine of the 13 collections, B351 to eight, B405 to seven, B580 to four, and Dohadak to six; whereas Reno was susceptible to all collections. The collections reported were selected as representative of each of the 13 groups of collections listed in Table 3. In further inoculation studies, each of these collections would be used as representative of the group of collections from which it was selected.

Reaction of Winter Barley Varieties to the Thirteen Representative Collections.—It has been shown that the 34 collections of *U. nuda* can be divided into 13 physiologic groups on the basis of their pathogenicity on the six different winter barley varieties. Having selected a single

TABLE 4 -- PERCENT INFECTION AND REACTION OF SIX DIFFERENTIAL VARIETIES OF WINTER BARLEY TO EACH OF THIRTEEN COLLECTIONS OF USTILAGO NUDA

Variety	C. I. Number	Percent Infection with Collection Number:												
		1	2	4	5	9	10	11	14	15	17	25	29	35
North Carolina Hooded 26	7026	1	1	5	6	1	1	1	13	9	15	14	2	34
B351		19	7	2	11	6	19	4	5	2	5	1	14	17
B405		12	4	9	16	49	4	45	16	1	56	1	5*	6
B580	7567	21	14	6	8	48	9	60	13	2	69	16	18	26
Dohadak	5187	18	5	5	6	20	35	4	49	17	0	29	0	40
Reno	6561	55	63	70	47	63	44	46	48	24	62	41	59	53

*1 year's results only.

Variety	C. I. Number	Reaction to Collection Number:												
		1	2	4	5	9	10	11	14	15	17	25	29	35
North Carolina Hooded 26	7026	R	R	R	R	R	R	R	S	R	S	S	R	S
B351		S	R	R	R	R	S	R	R	R	R	R	S	S
B405		S	R	R	S	S	R	S	S	R	S	R	R	R
B580	7567	S	S	R	R	S	R	S	S	R	S	S	S	S
Dohadak	5187	S	R	R	R	S	S	R	S	S	R	S	R	S
Reno	6561	S	S	S	S	S	S	S	S	S	S	S	S	S

R = Resistant; S = Susceptible.

collection representative of each of these physiologic groups, it then was feasible to study the reactions of the other varieties and experimental strains that were inoculated during the course of the investigation. Table 5 gives the average percent infection in the varieties and the strains of winter barley obtained when they were inoculated with a representative collection from each of the 13 physiologic groups of collections of *U. nuda.*

The varieties and selections have been classified into seven groups according to their origin, lemma appendage, and commercial use. They will be discussed within this grouping.

I. *Rough-awned, Tennessee winter type:* This group includes the most productive and most winter-hardy barley varieties grown in Missouri at the beginning of this study. At that time Reno was the leading variety in productivity and in winter hardiness, followed closely by Kentucky 1, Ward, and Michigan Winter. All varieties in this group were highly susceptible to the collections of *U. nuda* used.

II. *Smooth-awned varieties of Tennessee Winter type:* Only one variety, Smooth-awn 86, was tested. It proved highly susceptible to all collections with which it was inoculated.

III. *Introduced commercial varieties:* These varieties were found susceptible to all collections with which they were inoculated.

IV. *Selections from composite crosses:* Davidson, a rough-awned variety, gave a varied reaction to the collections used. It was resistant to the group of collections represented by collections 2, 10, 14, and 29, and varied a great deal in the degree of susceptibility to the remaining groups. However, this variety was difficult to study, as a wide variation in the percentage of smutted heads was observed in consecutive years. Nassau, a smooth-awned variety, was susceptible to all except collection 25. On the basis of one year's data, Harbine appeared to be resistant to collections 25 and 35. However, it was susceptible to collections 2 and 11. Kenbar was resistant to collection 2 and susceptible to 11, 25 and 35 in one year's results only.

V. *Hooded varieties of Tennessee Beardless or similar origin:* This group of varieties was an excellent source of resistance to many of the collections of *U. nuda.* The varieties Hooded 16 and B467 were resistant to all collections except 35. North Carolina Hooded 26 was resistant to all collections except 14, 17, 25, and 35, and only weakly susceptible to collections 14, 17, and 25. B351 was susceptible to collections 1, 10, 29, and 35, and resistant to all others. However, this variety was not highly susceptible to any of the collections. Although B405 was susceptible to the smut collections 1, 5, 9, 11, 14, and 17, it was resistant to the most virulent of all collections, number 35. Tucker was resistant to collections 9, 11, and 17 only. Other varieties in this group were resistant to many of the collections of *U. nuda* with which they were inoculated. B355, a selection from Missouri Early Beardless, was susceptible to all collections with which it was inocu-

TABLE 5 -- PERCENT INFECTION OBTAINED IN VARIETIES AND EXPERIMENTAL STRAINS OF WINTER BARLEY WHEN INOCULATED WITH THIRTEEN REPRESENTATIVE COLLECTIONS OF USTILAGO NUDA

Variety	C. I. Number	Percent Infection With Collection Number: 1	2	4	5	9	10	11	14	15	17	25	29	35
I. Rough-Awned Tennessee winter type:														
Michigan Winter	2036	39	63	40	51	68	71							
Purdue 21	4581	71	90	63	82	56	43							
Kentucky 6	4678	47	34	42	23	32	28	31*	63*	28*	26*	18	24	
Ward	6007	48	78	67	64	80	58	84	68	51				
Kentucky 1	6056	68	31	28	53	13	63	23	59	52	30		20	
Reno	6561	55	63	70	47	63	44	46	48	24	62	41	59	53
II. Smooth-Awned varieties of Tennessee winter type:														
Smooth-awned 86	6668	43	85	91	51	71	96	74	82	63				
III. Introduced commercial varieties:														
Alaska	534	24	39	61	61	18	74							
Sunrise	6272	71	65	88	67	69	62							
Poland	6280	39	66	54	31	21	35	25	70*	24	41*	67	43	
Wong	6728	20	76	37	54	14	61	50	29	53				
IV. Selections from composite crosses:														
Davidson	6373	11	4	19	31	42	5	26	6	14	37	13	10	
Nassau	7022	43	34	42	16	60	26	48	38	40	57	9	19	
Harbine	7524		32*					37*				5*		2*
Kenbar	7574		2*					49*				20*		11*
V. Hooded varieties of Tennessee Beardless or similar origin:														
Hooded 16	6574	5	2	2	2	1	1	0	2	1	1	0	0*	46
N. C. Hooded 26	7026	1	1	5	6	1	1	1	13	9	15	14	2	34
Tucker	7039	40	17	22	50	8	61	4	54	31	5	14	23	
B351, M.E.B. Sel.		19	7	2	11	7	19	4	5	2	5	1	14	17
B355, M.E.B. Sel.		45	39	9	76	13	54						15	
B405, M.E.B. Sel.		12	4	9	16	49	4	45	16	1	56	1	5*	6
B411, M.E.B. Sel.		0*	2	0*	0*	1	0	0			0*			56
B495, M.E.B. Sel.			12			25					25			12
B502, M.E.B. Sel.		16*	1	1*	0*	1	0				0*			
B538, M.E.B. Sel.		45*	2	21*	7*	1	1				0*			
B467, Kinroku Sel.		8	2	1	1	3	0	1	4	2	1	5	0	78

TABLE 5 -- CONTINUED

Variety	C. I. Number	Percent Infection With Collection Number: 1	2	4	5	9	10	11	14	15	17	25	19	35
VI. Selections of Hybrid origin with Mo. Early Beardless parentage:														
Mo. B-400 (Ky. 5 x M.E.B.)	7568	30	20	53	30	52	23	73*	28*	21	92*	22*		
B575 Admire x M.E.B.	9168	2*	1	4*	1*	1	0	2			0*	7		48
B580 Admire x M.E.B.	7567	21	14	6	8	48	9	60	13	2	69	16	18	26
B588 Admire x M.E.B.		11*	5	9*	2*	29	2				90*			
B631 Admire x M.E.B.		15*	8	8*	24*	44	17				57*			
B635 Admire x M.E.B.		2*	1	9*	0*	35	6				56*			
B698 Admire x M.E.B.		3*	4	4*	2*	5	1				0*			
B699 Admire x M.E.B.	8064	3*	11	1*	16*	4	2	30			1*	51		51
B637 Mich. Winter x M.E.B.	7571		59					27				57		89
B696 Ky. 2 x M.E.B.		13	4	2	7	0	0	10			0	8		74
B640 Ward x M.E.B.	7572	0*	8	3*	0*	1	0	24			4*	21		77
B703 B289 x M.E.B.		3	9	2*	6	6	13	13			10*	0		40
B705 Ky. 5 x M.E.B.	9169		26					34				50		17
VII. Selections from U.S.D.A. World Collection:														
Han River, China	206	3*	8	1*	15	49	3				79*			
Omar, Hybrid	898	41*	16	82*	53	37	16				17*			
Clancy, Russia	1002	0*	15	61*	20	9	8				0*			
Abundio, Persia	1412		0		49	10*	61*				0*			
Caucasus	3358	0*	3	0*	71	12	1				0*			
Russia	4959	0*	0	0*	68	2	2*							
Russia	4966		0	0*	0*			0				0		0
China	5087	0*	14	0*	7*	19	34				0*			
China	5091	0*	0	0*	2	1	0	0			0*	0		45
Omugi, Korea	5144		0*			41						47		34
Hokudo, Korea	5176	33*	0	0*	0	0	0				0*			
Kada, Korea	5180	49*	0*	0*	0*	0*	20*				0*			
Saru, Korea	5185	15*	10	4*	0*	14	1				4*			
Dohadak, Korea	5187	18	5	5	20	4	35	49	17	0	6	29	0	40
Denrai, Korea	5225		28		33	13	22							
Dobaku, Korea	5238	21*	9	22*	12	11	18				11*			

*One year's data only.

Figure 6. Barley florets just prior to anthesis. Anthers have burst and the filament is enlongating to dehisce them. Each floret must be inoculated within a few hours of anthesis.

lated except collection 4; and B495, also a selection from Missouri Early Beardless, was susceptible to the four collections with which it was inoculated.

VI. *Selections of hybrid origin with Missouri Early Beardless parentage:* Mo. B-400 was susceptible to all collections with which it was inoculated. This reaction is perplexing since this variety displays excellent natural field resistance to loose smut. B696 was resistant to all collections used except 1 and 35. B580 was resistant to collections 4, 5, 10, and 15, and susceptible to all others used. Selection B575 was resistant to all collections used, except number 35. B640 was susceptible to collections 11, 25, and 35 and gave evidence of resistance to all others. In this group of varieties, only Mo. B-400, B637, and B705 were susceptible to all of the collections with which they were inoculated. However, many of the readings were based on one year's data and will require additional study.

VII. *Selections from the United States Department of Agriculture World Collection:* Varieties in this group displayed a great deal of variation in their reaction to collections of *U. nuda.* C. I. 5187 was the only variety inoculated with a large number of different collections. It was resistant to groups represented by collections 2, 4, 9, 15, 17, and 29.

Of particular interest in this group of varieties is the immune reaction of C. I. 4966 to collection number 35, as well as other collections with which it was inoculated. Many of the introduced varieties offer potential sources of resistant germ plasm, varying according to the variety and collection of the pathogen used.

Collections of *Ustilago nuda* With Outstanding Virulence—Sev-

eral of the collections of *U. nuda* produced high infections in certain varieties. Among the more virulent of these were collections 1, 5, 9, 17, and 35. The infection percentages produced by each of these collections in selected susceptible varieties are listed in Table 6.

Collection number 1 produced high infections in B351, B502, and B538, all selections from Missouri Early Beardless; and in Hokudo, an introduction from Korea. This collection was obtained from the variety Kentucky 1 in Mississippi. Collection 5 produced high infection in B405, a se-

TABLE 6 -- INFECTION PERCENTAGES IN DIFFERENTIALS AND VARIETIES SUSCEPTIBLE TO FIVE VIRULENT COLLECTIONS OF USTILAGO NUDA

Variety	C. I. Number	Percent Infection in Collection Number: 1	5	9	17	35
Some varieties susceptible to collection 1:						
*B351 M.E.B. Sel.		19	11	6	5	17
B502 M.E.B. Sel.		16	0	1	0	--
B538 M.E.B. Sel.		45	7	1	0	--
Hokudo, Korea		33	--	0	0	--
Some varieties susceptible to collection 5:						
*B405 M.E.B. Sel.		12	16	49	56	6
B699 Admire x M.E.B. Sel.	8064	3	16	4	1	51
Omar, Hybrid	898	4	53	37	17	--
Clancy, Russia	1002	0	20	9	0	--
Abundio, Persia	1412		49	10	0	--
Caucasus	3358	0	71	12	0	--
Russia	4959	0	68	2	--	--
Some varieties susceptible to collection 9:						
*B405 M.E.B. Sel.		12	16	49	56	6
*B580 Admire x M.E.B. Sel.	7567	21	8	48	69	26
B588 Admire x M.E.B. Sel.		11	2	29	90	--
B635 Admire x M.E.B. Sel.		2	0	35	56	--
Han River, China	206	3	15	49	79	--
China	5087	0	7	19	0	--
Some varieties susceptible to collection 17:						
*North Carolina Hooded 26		1	6	1	15	34
*B405 M.E.B. Sel.		12	16	49	56	6
*B580 Admire x M.E.B. Sel.	7567	21	8	48	69	26
B588 Admire x M.E.B. Sel.		11	2	29	90	--
B635 Admire x M.E.B. Sel.		2	0	35	56	--
Han River, China	206	3	15	49	79	--
Some varieties susceptible to collection 35:						
Hooded 16	6574	5	2	1	1	46
*North Carolina Hooded 26	7026	1	6	1	15	34
B411 M.E.B. Sel.		0	0	1	0	56
B467 Kinroku Sel.		8	1	3	1	78
B575 Admire x M.E.B. Sel.	9168	2	1	1	0	48
B640 Ward x M.E.B. Sel.	7572	0	0	1	4	77
B696 Ky. 2 x M.E.B. Sel.		13	7	0	0	74
B703 B289 x M.E.B. Sel.		3	6	6	10	40
China	5091	0	2	1	0	45
Dohadak, Korea	5187	18	20	4	6	40

*Denotes differential variety.

Figure 7. Missouri Agricultural Experiment Station selection B575 inoculated wth Collection 2. No infection.

Figure 8. Missouri Agricultural Experiment Station selection B575 inoculated with collection 35. Note heavy infection.

lection from Missouri Early Beardless, and in B699, a hybrid selection with Missouri Early Beardless parentage. High infection also was produced in Omar, of unknown hybrid origin, and several introductions from Russia and nearby areas. Collection 5 was obtained from Smooth-Awn 88 growing at Raleigh, N. C. Collection 9 produced high infection in B405, a selection from Missouri Early Beardless; in B580, B588, and B635, all hybrid selections with Missouri Early Beardless parentage; and in two introductions from China. Collection 17 appears similar in pathogenicity to collection number 9, except that one of the introductions from China, C. I. 5087, was resistant to collection 17 but susceptible to collection 9, and North Carolina Hooded 26 was susceptible to collection 17, but resistant to collection 9. Both collections 9 and 17 were obtained from the variety Davidson at Raleigh, N. C.

Collection number 35 was the most virulent of the collections used. Only B405, a Missouri Early Beardless selection; Harbine; and C. I. 4966, an introduction from Russia, were resistant. The reaction of B405 perhaps needs further testing, since all of the other hooded varieties and the hybrid selections were extremely susceptible to this collection. Collection 35 was the only collection that produced a susceptible reaction in B467, Kinroku selection; B411, Missouri Early Beardless selection; Hooded 16; and B575, Admire x Missouri Early Beardless selection. It was the only collection to produce high infection percentages in North Carolina Hooded 26; B696, Kentucky 2 x Missouri Early Beardless selection; and C. I. 5091, an introduction from China. Collection 35 was obtained from B467 growing in the barley breeding nursery at Columbia, Mo. Since most of the barley selections being developed at the Missouri Agricultural Experiment Station derive their resistance from Missouri Early Beardless, or from another of the hooded varieties, this collection potentially is very important in Missouri. So far it has not been collected from any other source. Harbine was resistant but this reaction was based on one year's data only. C. I. 4966 was immune to this collection, as it was to all other collections with which it was inoculated.

Varieties With Outstanding Resistance—Several varieties have shown excellent resistance to a large number of collections. These varieties and the infection percentages obtained when inoculated with each of 13 collections are given in Table 7. Included are the hooded varieties Hooded 16; North Carolina Hooded 26; B351 and B411, Missouri Early Beardless selections and B467, a Kinroku selection. One hybrid selection with Missouri Early Beardless parentage, B575, was resistant to all collections except number 35; and one, B696, was resistant to all except collections 1 and 35. C. I. 5091, an introduction from China, was resistant to all collections with which it was inoculated except number 35. Another introduction, C. I. 4966 from Russia, was immune to the five collections with which it has been inoculated, including number 35.

TABLE 7 -- INFECTION PERCENTAGES IN NINE VARIETIES WITH OUTSTANDING RESISTANCE WHEN INOCULATED WITH EACH OF 13 COLLECTIONS OF USTILAGO NUDA

Variety	C. I. Number	Percent Infection with Collection Number: 1	2	4	5	9	10	11	14	15	17	25	29	35
Hooded Varieties:														
Hooded 16	6574	5	2	2	2	1	1	0	2	1	1	0	0	46
N. C. Hooded 26	7026	1	1	5	6	1	1	1	13	9	15	14	2	34
B351 M.E.B. Sel.		19	7	2	11	7	19	4	5	2	5	1	14	17
B411 M.E.B. Sel.		0	2		0	1	0	0			0			56
B467 Kinroku Sel.		8	2	1	1	3	0	1	4	2	1	5	0	78
Selections of Hybrid origin with Missouri Beardless parentage:														
B575 Admire x M.E.B. Sel.	9168	2	1		1	1	0	2			0	7		48
B696 Ky. 2 x M.E.B. Sel.		13	4	2	7	0	0	10	1		0	8		74
Selections from U.S.D.A. World Collection:														
Russia	4966		0		0			0				0		0
China	5091	0	0		2	1	0	0			0	0		45

TABLE 8 -- DISTRIBUTION OF 34 COLLECTIONS OF USTILAGO NUDA FROM TEN STATES ACCORDING TO GROUP WITH SIMILAR PATHOGENICITY

State	Number and Percentage of Collection in Group Number: I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	Total Number of Collection
Missouri	1	3	4			3	1	1		1		1		15
Arkansas		1				1					1			3
Mississippi	1													1
Texas			1	1	2	1								5
North Carolina				1	1	1			1					4
New Jersey			1											1
West Virginia													1	1
Illinois				1										1
Kentucky					1			1						2
Nebraska				1										1
Total	2	4	6	4	4	6	1	2	1	1	1	1	1	34
Percentage	6	12	18	12	12	18	3	6	3	3	3	3	3	

Distribution by States of the Collections of *Ustilago nuda*--The 34 collections of *U. nuda* reported here were obtained from ten states. In Table 8 the distribution of the collections is reported by state and collection group (see Table 3). Groups I and VI and Group VIII all comprise more than one collection. In every case collections within these groups were obtained from more than one state. Three of the collection groups, II, IV, and VI, comprise collections obtained from four different states. Thus it would appear that these different collections are widely distributed over the winter barley region. The similarity of collections from different geographic areas may, in part, be a result of the manner in which many of the collections were obtained. Nearly all collections from outside Missouri were sent in by research workers at the various agricultural experiment stations. No doubt many of the collections were obtained from the barley breeding nurseries, among which there has been relatively free interchange of breeding materials. Pathogenic strains of the smut fungus within these breeding materials could in this manner be widely distributed. Two of the collections, 29 and 35, have been obtained only from the barley breeding nursery of the Missouri Agricultural Experiment Station.

DISCUSSION

Data have been presented to show the reaction of 58 varieties and selections of winter barley when artifically inoculated with 4 or more of 34 collections of *U. nuda* obtained from ten states. It has been shown that the 34 collections of *U. nuda* may be divided into 13 physiologic groups on the basis of their pathogenic reaction on 6 winter barley varieties. The immediate question then is whether the evidence will support the designation of these physiologic groups as specific physiologic races. It is the author's opinion that there is ample evidence of physiologic specialization but positive identification of specific races will require additional study. This conclusion is based on several considerations.

Figure 9. Missouri Agricultural Experiment Station selection B467 inoculated with collection 2. No infection.

Figure 10. Missouri Agricultural Experiment Station selection B467 inoculated with collection 35. Note heavy infection. Collection 35 was obtained from B467.

First, variation in infection percentages was obtained in different years' results. This was especially true in the varieties Kentucky 6, Davidson, Poland, and Tucker. Other investigators (26, 39, 45) also have experienced this difficulty. It is probable that the variation in infection percentages in different years was a result of the interaction of the host and fungus to the conditioning influences of the environment. The time of infection appears to be a critical period in the life cycle of the pathogen (9, 17, 18, 35, 39, 53, and 55). The optimum time for floral inoculation is shortly following anthesis. Inoculated florets frequently resulted in increased sterility, in reduced germination, and in reduced seedling vigor. If the florets were inoculated one to two days following anthesis, these adversities were minimized, and the percentage infection remained very near maximum. Excessive live-spore concentration in the inoculum also reduced the percentage of smutted heads, corroborating the finding of Zehner and Humphrey (55). This was minimized by reducing the live-spore concentration in the inoculum to 1/10 of 1 percent (22, 49). The relative humidity at the time of inoculation has been found to have a profound effect on the percent of infection obtained; low relative humidity results in low infection and high relative humidity results in high infection in susceptible material (16, 42). The amount of winter-kill and the possibility of variety mixtures in the material inoculated may have contributed to the apparent variability of infection percentage received from the same collection inoculated into the same variety in different years. Thren (49) has reported significant reductions in the percent stand resulting from inoculated seed over uninoculated seed, due to winter-kill. Hori (13) reported little smut following the late fall planting of barley in Japan.

Variability within the smut fungus also may be a factor in the variation in infection percentage. The rusts and powdery mildew can be propagated easily by their asexual spores and thereby true biotypes can be maintained. On the other hand, in the smut fungi the chlamydospores are the "diploid phase," a spore which cannot be propagated independently. Reduction and segregation and subsequent conjugation take place when these spores germinate. The conjugation of the haploid lines of opposite sex form the dikaryophase in the smut life cycle. Therefore conjugation must take place prior to infection of the suscept and before chlamydospores are again produced. Consequently, each new generation of chlamydospores may consist of a new group of related biotypes (4). For these reasons and due to incomplete evidence presented here, the term "physiologic group " is used instead of "physiologic race" to describe collections of chlamydospores having the same relative virulence on certain possible differential varieties.

Second, a careful consideration should be given to the selection of differentials. Are the differentials used here the most suitable that could be selected? Perhaps the most useful differentials would be those varieties that are used as resistant parental material in breeding programs. Since the

authors are interested in breeding winter barleys, and since spring type varieties do not grow satisfactorily in this climate, only winter types were used. Of the six differential varieties used to separate the 34 collections of *U. nuda* into physiologic groups, four apparently derived their resistance from a similar source. These were North Carolina Hooded 26; B351 and B405, Missouri Early Beardless selections; and B580 from the cross Admire x Missouri Early Beardless selection. One differential, Dohadak, was introduced from Korea. The sixth variety, Reno, was used only as a susceptible check. It is quite possible that strains as closely related genetically as B351, B405, B580, and perhaps North Carolina Hooded 26, would serve to differentiate closely related biotypes of the smut fungus only. Also, hooded varieties are quite susceptible to frost damage, and winter-killing within these varieties has reduced the stands at Columbia so that limited data was obtained in certain years. More winter hardy differential varieties could, in all probability, lead to more reliable results.

Regardless of these limitations, the value of grouping together the 34 collections on the basis of their pathogenicity into physiologic groups is readily recognized as a preliminary step to a more accurate classification of physiologic races. It has been used here to study the resistance of other varieties. The reactions of each of 58 varieties to a representative collection of 4 or more of these 13 physiologic groups are reported. The study disclosed that the awned winter barley varieties of commercial importance in the United States are susceptible to a great number of collections of this smut. Hooded varieties of Tennessee Beardless origin, with the exception of Tucker and B355, were resistant to a very large number of collections. B467 was resistant to all collections except 35, and North Carolina Hooded 26 was susceptible to only four collections. A number of the bearded hybrid selections from the Missouri Agricultural Experiment Station were resistant to a large number of collections. This is of special interest, since a selection from the hooded variety Missouri Early Beardless was used as one of the parents in each of these crosses. B575 is an excellent example of a superior bearded hybrid which was resistant to all collections except 35. One introduction from Korea, C. I. 5187, also was resistant to a large number of collections. Data has been presented on the reaction of a number of additional hybrid selections and on strains from the U. S. D. A. World Collection to selected collections of the pathogen. It would appear from these data that a great many varieties of varied origin are available as sources of resistance to *U. nuda* (Table 5).

When this study was initiated, information was available regarding the reaction of many of the barley strains to a single local collection of *U. nuda* (26, 27, 28). Information was not available on the reaction of these varieties to varied collections of the organism which might differ in their pathogenicity. It was not known whether varieties resistant to a local collection of *U. nuda* would maintain their resistance when grown in winter barley areas

Figure 11. Reno barley inoculated with collection 2. Note heavy infection.

Figure 12. C. I. 4966 barley inoculated with collection 35. Note no infection from the inoculation.

of other states, or even in separated areas in Missouri where different races of this organism might exist. Since breeding for resistance to disease involves specific evaluation of the response of the experimental varieties, not to one collection only, but to a representative sample of many collections of the pathogen, the grouping of the collections into physiologic races is of great practical value (48). This permits the testing of a maximum number of varieties with a minimum number of different collections, while still retaining the wide degree of "possible infection" necessary in establishing resistance to the disease under varied conditions. The 34 collections of *U. nuda* have been grouped into 13 physiologic groups on the basis of their pathogenic reaction on 6 winter barley varieties. By using these 13 possible physiologic races, it should be possible to obtain the same relative information regarding the degree of resistance or susceptibility of a variety as has been obtained by the use of 34 collections. The value of this grouping for testing breeding material not yet inventoried in a plant breeding program is readily apparent.

Setting up the line of demarcation between resistance and susceptibility was purely arbitrary, although it had some precedents. Bever (1) used 10 percent and below as resistant in differentiating races of *U. tritici.* Rodenhiser and Holton (34) used 0-10 percent as a resistant reaction in their studies on Races of bunt in wheat. Shands and Schaller (39) designated spring barley varieties resulting in 5 percent infection of *U. nuda* and below as resistant. Poehlman (26) used three infection classes in discussing resistance of winter barley to *U. nuda:* 5 percent and under, resistant; 5.1 to 17 percent, intermediate; and over 17 percent, susceptible.

Data indicate that many of the hooded strains of winter barley and the hybrid selections of winter barley deriving their loose smut resistance from Missouri Early Beardless selections are a rich source of germ plasm resistant to loose smut collections from a wide geographic area. North Carolina Hooded 26; Hooded 16; Missouri Early Beardless selection B351; Kinroku selection B467; B575, Admire x Missouri Early Beardless selection; and C. I. 4966, an introduction from Russia, all possess excellent resistance. The resistance of the B575, is of special interest since this strain is being increased for possible distribution by the Missouri Agricultural Experiment Station. B575 was resistant to all of the "physiologic groups" with which it was inoculated, except collection number 35.

Collection number 35 merits special consideration since all of the hooded varieties, except Missouri Early Beardless selection B405, and all of the hybrid strains with Missouri Early Beardless parentage were extremely susceptible to this collection. It also is important because B575, which is being increased for distribution, is susceptible only to this collection. Special precautions are being taken to isolate the smut nursery from the breeding nursery, due to the extreme virulence of collection 35. While this collection

was picked up in our own breeding nursery in 1950, no collection with similar pathogenicity has been isolated since. Crosses already have been made between B575 and C. I. 4966, a variety which thus far has been resistant to collection number 35.

SUMMARY

This study shows the reactions of 58 varieties and selections of winter barley when artificially inoculated with 4 or more of 34 collections of *Ustilago nuda* obtained from 10 states. The winter barley varieties and selections originated from varied sources in this country and the U. S. D. A. World Collection. The 34 collections of *U. nuda* were divided into 13 physiologic groups on the basis of their pathogenic reaction on 6 of the winter barley varieties.

The rough-awned Tennessee winter type varieties, the smooth-awned Tennessee winter type varieties, and the introduced commercial varieties commonly grown in the United States were susceptible to all collections of *U. nuda* with which they were inoculated.

Hooded varieties of Tennessee beardless and similar origin provided an excellent source of resistance to many of the *U. nuda* collections. Selections from the variety Missouri Early Beardless were resistant to a great number of collections of the pathogen.

Selections of hybrid origin with Missouri Early Beardless parentage varied in their response according to the collection and selection used. Several were excellent sources for resistant parental material.

The varieties from the U. S. D. A. World Collection varied in their reaction to collections of the pathogen but many offered potential sources of resistant germ plasm, varying according to the variety and collection of the pathogen used. It would appear from the data presented that a great many winter barley varieties of varied origin are available as sources of resistance to *U. nuda.* Resistant varieties were: North Carolina Hooded 26; Hooded 16; Missouri Early Beardless selection B351; Kinroku selection B467; B575, Admire x Missouri Early Beardless selection; C. I. 4966, an introduction from Russia; and B696, Kentucky 2 x Missouri Early Beardless selection.

BIBLIOGRAPHY

1. Bever, Wayne M. 1947. Physiologic Races of *Ustilago tritici* in the Eastern Soft Wheat Region of the United States. Phytopath. 37:889-895.
2. Bever, Wayne M. 1953. Futher Studies on Physiologic Races of *Ustilago tritici.* Phytopath. 43:681-683.
3. Brefeld, Oscar. 1912. Investigations in the General Field of Mycology. Continuation of the Moulds and Yeasts. Part XIII, Smut Fungi (Hemibasidia IV) Munster. English translation by Frances Dorrance.
4. Christensen, J. J. and H. A. Rodenhiser. 1940. Physiological Specialization and Genetics of the Smut Fungi. The Botanical Review. 6:389-425.
5. Christensen, J. J. and E. C. Stakman. 1926. Physiologic Specialization and Mutation in *Ustilago zeae* (Beck) Ung. Phytopath. 16:979-999.
6. Cloninger, C. K. 1951. The Reaction of Winter Barley Varieties to Collections of *Ustilago nuda,* (Jens.) Rostr. Ph. D. Thesis, University of Missouri.
7. Compton, LeRoy E. and Ralph M. Caldwell. 1946. Yield Reductions Caused by Loose Smut of Wheat. Phytopath. 36:1040-1042.
8. Faris, James A. 1924. Physiologic Specialization of *Ustilago hordei.* Phytopath. 14: 537-557.
9. Freeman, E. M. and Johnson, Edward C. 1909. The Loose Smuts of Barley and Wheat. U. S. D. A. Bur. Plant Indus. Bul. 152.
10. Hanna, W. F. 1937. Physiologic Forms of Loose Smut of Wheat. Canadian Jour. Res. Sect. C. Bot. Sci. 15:141-153.
11. Holton, C. S. and H. A. Rodenhiser. 1942. New Physiologic Races of *Tilletia tritici* and *T. levis.* Phytopath. 32:117-129.
12. Holton, C. S. and H. A. Rodenhiser. 1948. Physiologic Specialization in the Oat Smut Fungi and Its Relation to Breeding Oats for Smut Resistance. U. S. D. A. Tech. Bul. 952.
13. Hori, S. 1907. Seed Infection by Smut Fungi of Cereals. Bul. Imp. Cent. Agr. Exp. Sta. Japan. 1:163-176.
14. Johnston, T. H. 1953. Inheritance of Resistance to Certain Races of Loose Smut. *Ustilago nuda* (Jens.) Rostr. in Winter Barley. Ph.D. Thesis, Iowa State College.
15. Leben, Curt and D. C. Arny. 1954. Soaking Treatments for the Control of Loose Smut of Barley. Phytopath. 44:329-330.
16. Mackie, W. W. 1931. Diseases of Grain and Their Control. Calif. Agr. Exp. Sta. Bul. 511.
17. Maddox, Frank. 1896. Smut and Bunt. Agricultural Gazette of Tasmania. 4:92-95.
18. Middleton, G. K. and W. H. Chapman. 1941. Resistance to Floral-infecting Loose Smut *(Ustilago nuda)* in Fall Sown Barley Varieties at Statesville, North Carolina, Phytopath. 31:351-353.
19. Mooers, C. A. 1915. The Small Grains in Tennessee. Tennessee Agricultural Experiment Station Bulletin 112.
20. Moore, M. B. 1936. A Method for Inoculating Wheat and Barley with Loose Smuts. Phytopath. 26:397-400.
21. Nahmmacher, Jurgen. 1932. Bietrag zur Immunitatszuchtung der Gerste gegen *Ustilago nuda* Forma Spec. *hordei.* Phytopath. Zeitschr. 6:597-630 (R. A. M. 11:710.)
22. Oort, A. J. P. 1939. Inoculation Experiments with Loose Smuts of Wheat and Barley *(Ustilago tritici* and *U. nuda).* Phytopath. 29:717-728.
23. Oort, A. J. P. 1947. Stuifbrand Specialisatie, een Probleem vor den Kweker, Onderzoekingen over Stuifbrand, III. (Specialization of Loose Smut of Wheat, a Problem for the Breeder. Researches on Smut, III). Tijdschrift over Plantenziekten. 53:25-43.

24. Piekenbrock, P. 1927. Untersuchungen uber das Verhalten des *Ustilago tritici* an Sorten und Kreuzungen. (Investigations on the Behavior of *Ustilago tritici* towards Varieties and Hybrids.) Kuhn-Arch. 15:411-456. (Abstract in Zeitschr. fur Pflanzenkrankh. u. Pflanzenschultz, xxxviii, 3-4, p. 104, 1928). (R. A. M. 7:435:1928).
25. Poehlman, J. M. 1945. A Simple Method for Inoculating Barley with Loose Smut. Phytopath. 35:640-644.
26. Poehlmn, J. M. 1947. Sources of Resistance to Loose Smut, *Ustilago nuda,* in Winter Barleys. Jour. Am. Soc. Agron. 39:430-437.
27. Poehlman, J. M. 1949. Agronomic Characteristics and Disease Resistance of Winter Barleys Tested in Missouri, 1943 to 1948. Mo. Res. Bul. 442.
28. Poehlman, J. M. 1949. Sources of Resistance to Loose Smut, *Ustilago nuda,* in Winter Barleys of Foreign Origin. Jour. Am. Soc. Agron. 41:191-194.
29. Reed, George M. 1924. Physiologic Races of Oats Smuts. Am. Jour. Bot. 11:483-492.
30. Reed, George M. 1935. Physiologic Specialization of the Parasitic Fungi. Botanical Review. 1:119-137.
31. Reed, George M. 1940. Physiologic Races of Oats Smuts. Am. Jour. Bot. 27:135-143.
32. Rodenhiser, H. A. 1928. Physiologic Specialization in Some of the Cereal Smuts. Phytopath. 18:955-1003.
33. Rodenhiser, H. A. and C. S. Holton. 1937. Physiologic Races of *Tilletia tritici* and *Tilletia levis.* Jour. Agr. Res. 55:483-496.
34. Rodenhiser, H. A. and C. S. Holton. 1945. Distribution of Races of *Tilletia caries* and *Tilletia foetida* and Their Relative Virulence on Certain Varieties and Selections of Wheat. Phytopath. 35:955-969.
35. Ross, J. G., Wm. Semeniuk, D. K. Taylor, and B. C. Jenkins. 1948. Factors affecting the Degree of Infection of Barley by Loose-Smut (*Ustilago nuda* (Jens.) Rostr.). Sci. Agric. 28:481-492.
36. Sampson, Kathleen. 1925. Some Infection Experiments with Loose and Covered Smuts of Oats which Indicate the Existence in Them of Biological Species. Ann. Appl. Biol. 12:314-325.
37. Seiffert, Johannes. 1926. Kunstliche Bluteninfektionen zur Untersuchung der Empfanglichkeit Verschiedener Gerstensorten fur *Ustilago hordei nuda* und der Einfluss Eusserer Bedingungen auf die Hohe des Brandprozentes. Kuhn Archiv. 12:423-515. (R. A. M. 6:412. 1927).
38. Semeniuk, Wm. and J. G. Ross. 1942. Relation of Loose Smut to Yield in Barley Can. Jour. Research 20:491-500.
39. Shands, H. L. and C. W. Schaller. 1946. Response of Spring Barley Varieties to Floral Loose Smut Inoculation. Phytopath. 36:534-548.
40. Shands, R. G. 1951. Varietal Reaction to Trebi Loose Smut. Barley Worker's Conference, Madison, Wisconsin, January, 1951, page 33.
41. Stakman, E. C. 1926-1927. Racial Specialization in Plant Disease Fungi. Plant Pathology and Plant Physiology in Relation to Man. (Mayo Foundation Lectures 1926-1927). 207 pp. W. P. Saunders Co., Philadelphia.
42. Tapke, V. F. 1931. Influence of Humidity on Floral Infection of Wheat and Barley by Loose Smut. Jour. Agr. Res. 43:503-516.
43. Tapke, V. F. 1937. Physiologic Races of *Ustilago hordei.* Jour. Agr. Res. 55:683-692.
44. Tapke, V. F. 1943 Occurrence, Identification, and Species Validity of the Barley Loose Smuts. *Ustilago nuda, nigra,* and *medians.* Phytopath. 33:194-209.
45. Tapke, V. F. 1943. Physiologic Races of *Ustilago nigra.* Phytopath. 33:324-327.
46. Tapke, V. F. 1945. New Physiologic Races of *Ustilago hordei.* Phytopath. 35:970-976.

47. Taylor, J. W. and M. G. Zehner. 1930. The Effect of a Seed Disinfectant on Grain and Straw yields and Smut Control in Winter Barley. Jour. Amer. Soc. Agron. 22: 113-123.
48. Tervet, Ivan W. 1940. Problems in the Determination of Physiologic Races of *Ustilago avenae* and *U. levis*. Phytopath. 30:900-912.
49. Thren, Robert. 1938. Kritische Versuche zur Reisstenzprufung der Gerste gegan Flugbrand (*Ustilago nuda* (Jens.) Kellerm, et Sw.). Kuhn Archiv 44:211-231. (R. A. M. 17:516. 1938).
50. Thren, Robert. 1941. Zur Frage der physiologischen Specialisierung des Gerstenflugbrands *Ustilago nuda* (Jensen) Kellerm, et Sw. und der Entstehung never Gerstenbrandrassen. Phytopath. Zeithschr. 13:539-571.
51. Tulasne, L. R. and Charles Tulasne. 1847. Memoire sur les Ustilaginees Comparees aux Uredinees. Ann. Sci. Nat. Bot. III (2):12-126.
52. Tyner, L. E. 1953. The Control of Loose Smut of Barley and Wheat by Spergon and by Soaking in Water at Room Temperature. Phytopath. 43:313-316.
53. Vanderwalle, R. 1932. Contribution a l'etude des maladies charbonneuses de l'orge. Bull. Inst. Agron. et Sta. Rech. Gembloux. 1:291-322.
54. Wells, S. A. and A. W. Platt. 1949. The Effect of Loose Smut on Viability of Artifically Inoculated Barley Seeds. Sci. Agric. 29:45-52.
55. Zehner, Marion Griffiths and Harry B. Humphrey. 1929. Smuts and Rusts Produced in Cereals by Hypodermic Injection of Inoculum. Jour. Agr. Res. 38:623-627.