THE INHERITANCE OF LOOSE SMUT REACTION IN A CROSS BETWEEN TWO SPRING WHEATS, REDMAN AND THATCHER

by

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INTRODUCTION

The losses due to loose smut of wheat, Ustilago tritici (Pers.) Rostr., are relatively minor in comparison with some of the more destructive diseases, but loose smut is, nevertheless, quite general throughout the world. Heald (7)* states that it is found wherever wheat is grown, but adds that in many environments it is not sufficiently abundant to be an important factor in wheat production. According to 1927 New Zealand statistics, loose smut of wheat causes an average loss of 2 per cent, but this figure may be increased to 50 per cent when the disease occurs in epidemic proportions (1). Tapke (18) reported that during the ten year period 1917-1926 loose smut in the United States took an estimated toll of wheat which averaged more than 10,000,000 bushels annually. In the states adjoining Canada the comparable figures were; for North Dakota, above 1,000,000 bushels; for Montana and Minnesota, between 250,001 and 500,000 bushels each. Dickson (3) classifies the disease as severe in the soft red winter and hard red spring wheat areas of Central and Eastern North America.

Hanna and Popp (6) reported counts of loose smut made in fifteen fields of Reward wheat in different districts of Manitoba in 1930. The average infection was 2.2 per cent, while in one of these fields it was 7.6 per cent. Compton and Caldwell (2) found that percentage of infection is closely correlated with resulting losses in

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* Numbers in parenthesis refer to Literature Cited.

yield. The control of this disease is therefore of considerable importance.

Loose smut infection is intra-seminal and, as a result, the disinfection methods in common use for surface-borne spores are of no value. Effective treatment involves heating the seed* to such a degree that the internal mycelium is killed, but that the embryo is not. This is usually accomplished by treatment with hot water, as outlined by Heald (7), and many others. This treatment is not practicable for the average farmer with his limited supply of equipment, and for this reason is not practised to any great extent.

The ideal method of control is the breeding of varieties which are highly resistant or immune to loose smut. Some work has already been done toward this objective, and resistant varieties are available for use as parental material. However, the organizing of an efficient breeding program would be greatly aided by a knowledge of the mode of inheritance of loose smut resistance.

It was with this end in view that the present study was undertaken. Use was made of two varieties which are at present being used as parental material in the breeding program being carried on at the Dominion Laboratory of Cereal Breeding at Winnipeg. One of these varieties is highly resistant to loose smut, while the other is only moderately so.

Another aid to an efficient breeding program would be the development of a quick method of determining the reaction of a plant to

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* Throughout this report seed refers to a caryopsis.

loose smut. The method in general use at present involves inoculating the plant with loose smut spores at anthesis, and later growing its progeny to maturity. This takes about six months and requires a large amount of greenhouse space. Any reduction in this time could materially shorten the time necessary to produce a new resistant variety.

In view of this situation it was decided that a more rapid method, which has recently been proposed, would be carried on in conjunction with the established method. An evaluation of this new method could then be made.

REVIEW OF LITERATURE

A number of investigators have carried out studies regarding the inheritance of resistance to loose smut. Piekenbrock (11) crossed the variety Grüne Dame with both Rümker's Dickkopf and Rimpau'd Roter Schlandstedt summer wheats. The first was completely resistant and the last two highly susceptible to a local collection of Ustilago tritici. He found that immunity segregated in the F_2 and F_3 generations and that it was inherited recessively. This conclusion was later confirmed by Grevel (5).

Kilduff (8) made genetic studies of the F_2, F_3, and F_4 progeny of two common wheat crosses, Kota x Red Bobs, and Kota x Garnet. Kota was classed as susceptible, Red Bobs as immune, and Garnet as resistant, to the collection of loose smut used. Because of a lack of agreement in infection percentages in the F_3 and F_4, Kilduff could not offer any hypothesis to explain his findings. He suggested that some factor of a non-physiological order might be involved in the different degrees of resistance exhibited by two of the parents.

Roemer (12), in an account of 14 years of breeding work carried on at the Halle Agricultural Institute in Germany, stated that resistance to loose smut was recessive and dependent upon a single Mendelian factor.

Rudorf and Rosenstiel (13) artificially inoculated the F_2 plants of a cross between the susceptible variety San Martin and the resistant one 38 M.A., and studied the behavior of the F_3 generation.

The indications were that the resistance of 38 M.A. probably depended on three recessive factors.

Tingey and Tolman (19) investigated the inheritance of loose smut resistance in three crosses: Hope x Federation, Hope x Dicklow No.3, and Preston x 01-24. Hope appeared to be immune, Preston highly resistant, 01-24 resistant, Dicklow No.3 susceptible, and Federation highly susceptible to the inoculum used. Their results indicated that at least three factors were involved in the inheritance of resistance. The degree of resistance of a variety depended on the number of these factors present in it.

Larose and Vanderwalle (9) made reciprocal crosses between the highly susceptible variety Vilmorin 27 and the resistant variety Jubile, and at the same time inoculated the female plants with smut spores from infected plants of the susceptible variety. In neither case did any of the seeds give rise to smutted F_1 plants.

Methods of determining if loose smut mycelium is present in the embryos of seeds have been presented by Skvortzoff (17), Simmonds (15), and W. Popp* (unpublished). Vanderwalle (20), in a comparative study of experimentally infected embryos from the seeds of susceptible and resistant varieties, showed that the embryo of the latter alone was unaffected. Popp, on the other hand, found mycelium in the embryos of resistant varieties.

Saburova (14) states that loose smut infection in young wheat

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* Assistant Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg.

plants may be diagnosed by the presence of certain abnormalities in the rudimentary ear.

No references were found concerning methods similar to the Seedling Examination Method used herein.

MATERIALS AND METHODS

Varieties and Hybrids

The wheat varieties used in this investigation are important parental material in breeding work carried on at the Dominion Laboratory of Cereal Breeding at Winnipeg. They are :

Redman Selection	R.L. 1834.7*	
Thatcher	R.L. 1945	C.A.N. 1820**

Redman Selection was obtained from the variety Redman, R.L. 1834.1, C.A.N. 3633. Redman itself originated from a cross between Regent and Canus and is moderately resistant to loose smut (16).

Thatcher originated from the cross (Marquis x Iumillo) x (Marquis x Kanred). It is highly resistant to loose smut (16).

Two F_1 plants from a cross of Redman Selection by Thatcher were taken at random. The seeds from each of these plants were space sown in a plot in the field. Three plants of each parent were also taken at random, and the seeds from these plants were sown in separate plots adjacent to those containing the hybrid material.

It was necessary to determine the reaction of F_2 plants on the basis of the performance of their F_3 progeny in order to ensure that the ratio of resistant to susceptible F_2 plants would not be influenced by

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* Dominion Laboratory of Cereal Breeding Accession Number.

** Canadian Accession Number.

escapes which were classified as resistant.

Inoculation Procedure

During anthesis several heads on each of about 120 plants of the hybrid F_2, and of about 30 plants of each parent were inoculated with loose smut. Some heads of Reward wheat, a susceptible variety, were also inoculated to provide a check on the efficiency of inoculation.

The inoculum used was provided by W. Popp of the Dominion Laboratory of Plant Pathology at Winnipeg and was designated Ustilago tritici Physiologic Race 1.

Inoculations were made by the vacuum spore-suspension method devised by Moore (10).

When the inoculated plants had matured they were harvested and threshed individually.

Mature Plant Examination

Due to adverse conditions, it was found that only 94 of the F_2 plants had yielded enough seed to grow a representative F_3 line -- thirty to thirty-five seeds were considered adequate. It was decided that 20 lines of each parent would be indicative of the parental reactions. Six smaller lines of Reward were included to give an indication of the efficiency of inoculation.

Subsequently all these lines were grown in beds in two greenhouse sections. In order to make conditions comparable half of the material was grown in each section. The lines were distributed as shown in

Table I. Each line was grown in a separate row. The seed was treated with Ceresan in order to give protection against bunt and root rot infection.

After the material had grown to maturity, thirty plants were taken at random from each line, in order to obtain lines of uniform size. There were a few lines containing less than thirty plants. A count was then made of the number of smutted plants present in each line. Those lines containing no smutted plants were classified as resistant, the remaining lines as susceptible. The purpose in obtaining a uniform number of plants per line was to permit the correction of the expected genetic ratio for the size of the lines. However, this correction proved to be negligible and was therefore not applied.

The Chi-square test for goodness of fit as described by Goulden (4) was used to compare the observed ratio with the theoretical.

Embryo Examination

The object of the second phase of this investigation was to determine if either the presence or the amount of loose smut mycelium in the embryos of seeds was any indication of their susceptibility. This work was carried on with several of the Redman Selection and Thatcher lines. Ten embryos were examined in each line. The method used was developed by W. Popp. It is outlined in the Appendix.

Seedling Examination

In the third part of this investigation lines were classified

Table I

TOTAL NUMBER OF LINES AND THEIR
DISTRIBUTION BETWEEN GREENHOUSE SECTIONS

Plot No.	Variety or Hybrid	Number of Lines Grown in Section :		Total Number of Lines	
		A.	B.		
1	Redman Sel. x Thatcher	19	19	38	94
2	"	28	28	56	
3	Redman Selection	3	3	6	20
4	"	4	4	8	
5	"	3	3	6	
8	Thatcher	3	3	6	20
9	"	3	4	7	
10	"	4	3	7	
11	Reward	3	3	6	

as resistant or susceptible to loose smut by a method which was also developed by W. Popp. This method has the advantage of being much more rapid than that of growing the lines to maturity.

The material used was some of the F_3 and parental lines which had already been tested for loose smut reaction by the Mature Plant Examination Method. The number of lines tested is shown in Table II.

Each line was treated individually. Ten to twelve seeds were sown about two inches deep in a six-inch pot, after being treated with Ceresan to give protection against bunt and root rot infections. When the seedlings reached the two-leaf stage they were carefully dug up and washed. The coleoptile was peeled from each seedling to reveal the tillering node. A section of the mesocotyl about one-half inch long, which included the tillering node, was cut from each. These sections were first boiled until fairly transparent (three to five minutes) in a potassium hydroxide solution*, then boiled in water for two to three minutes, and finally boiled in a staining solution* for four to five minutes. After mounting in a special medium*, the sections were examined under a microscope. Sections in which loose smut hyphae were observed at or above the tillering node were classified according to the amount of mycelium present. An abitrary scale running from 0 to 8 was used: 0 indicating no infection; 8, very heavy infection. A line in which any section showed more than a trace of mycelium (i.e., classed as 2 or higher) was designated susceptible; the other lines, resistant.

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* See Appendix for formulae of solutions.

Table II

NUMBER OF LINES TESTED BY THE SEEDLING EXAMINATION METHOD

Plot Number	Variety or Hybrid	Number of Lines	Totals
1	Redman Sel. x Thatcher	25	49
2	"	24	
3	Redman Selection	4	8
4	"	2	
5	"	2	
8	Thatcher	2	6
9	"	3	
10	"	1	

The results obtained by this method were compared with those obtained by the Mature Plant Examination Method. The Chi-square test of independence and association (4) was applied to the resulting data. An additional comparison was made between the proportions of smutted plants obtained by the two methods. A correlation coefficient was calculated and a test of significance applied (4).

RESULTS AND DISCUSSION

Mature Plant Examinations

The reactions of the lines grown to maturity are shown in Table III. The lines of the check variety, Reward, were all classed as susceptible. As 72.7 per cent of the Reward plants were smutted, it was considered that the inoculations had been carried out efficiently.

The Thatcher lines contained no smutted plants, indicating immunity to Physiologic Race 1 of *Ustilago tritici*. However, since other tests of Thatcher conducted on a larger scale with the same physiologic race have shown an occasional trace of smut, this variety was considered to be nearly immune.

Redman selection showed only 21.9 per cent infection, indicating moderate resistance to loose smut. But, as this investigation was concerned with the Thatcher type of resistance or near immunity, lines containing any smutted plants were called susceptible. For purposes of genetic analysis, therefore, Redman Selection may be considered susceptible.

The F_3 lines of Redman Selection x Thatcher were classified as shown in Table III. The proportion obtained was 24 resistant to 70 susceptible lines, which closely approached the 1 : 3 ratio of 23.5 : 70.5. The Chi-square test of goodness of fit indicated that the deviation from the theoretical was not significant (P = .90). Tests of goodness of fit were also applied to the results from the individual plots in the different greenhouse sections and the same conclusion was

Table III

LOOSE SMUT REACTIONS OF LINES GROWN TO MATURITY

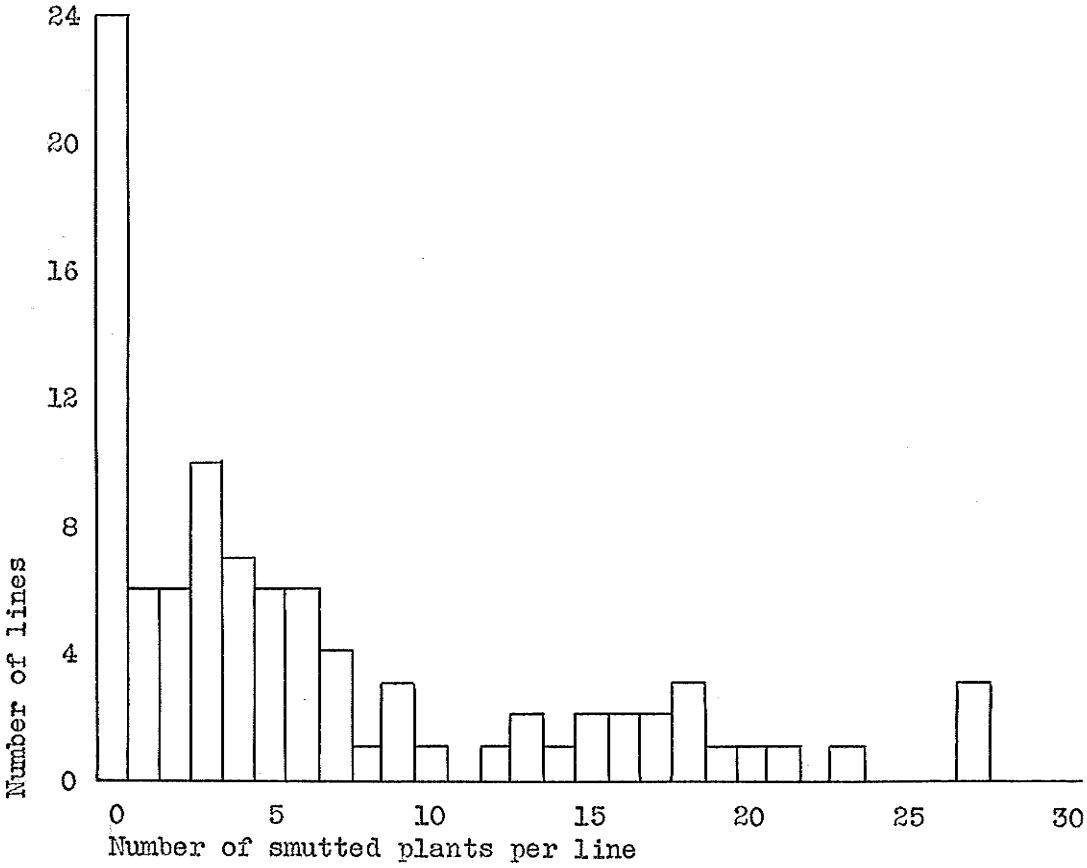
Plot No.	Variety or Hybrid	No. of Lines in Section: A. Resist	A. Susc.	B. Resist	B. Susc.	Totals Resist.	Totals Susc.
1	Redman Sel. x Thatcher	7	12	2	17	9 } 24	29 } 70
2	"	5	23	10	18	15 }	41 }
3	Redman Selection	0	3	0	3	0 } 0	6 } 20
4	"	0	4	0	4	0 }	8 }
5	"	0	3	0	3	0 }	6 }
8	Thatcher	3	0	3	0	6 } 20	0 } 0
9	"	3	0	4	0	7 }	0 }
10	"	4	0	3	0	7 }	0 }
11	Reward	0	3	0	3	0	6

reached. This indicated that a single major gene was responsible for the near-immunity of Thatcher.

The reaction of the lines is also displayed graphically. A histogram showing the distribution of the hybrid lines with regard to the number of smutted plants per line is given in Figure 1. A similar one for Redman Selection is shown in Figure 2.

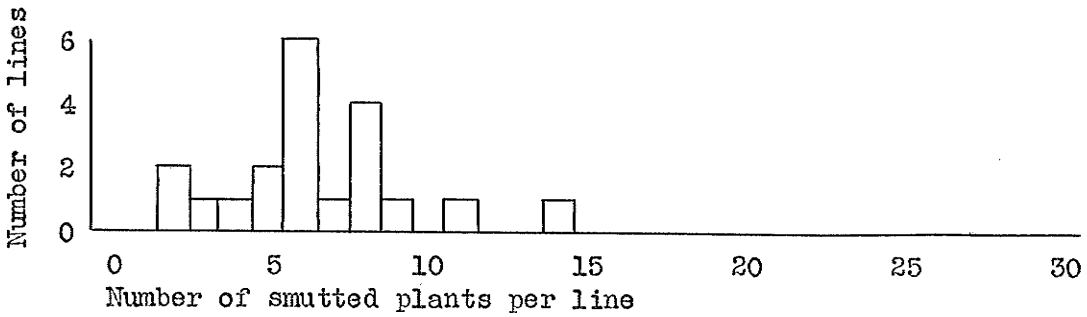
The distribution of the hybrid lines (Figure 1) showed a tendency to be divided into three groups: the first with no smutted plants, the second with one to ten, and the third with more than eleven smutted plants. These groups appeared to represent the resistant, segregating, and susceptible lines, respectively, which would be expected in an F_3 population. Hence the expected ratio, on the basis of a single factor, was 1 : 2 : 1 ; that is 23.5 : 47 : 23.5. The ratio obtained was 24 : 50 : 20. The Chi-square test of goodness of fit indicated that the deviation was not significant ($P = .73$). Again a single major gene for the near-immunity of Thatcher was indicated.

The loose smut reaction of the F_1 of Redman Selection x Thatcher was not tested, and thus no conclusion could be reached directly concerning the dominance or recessiveness of the Thatcher resistance. However, from the single major gene hypothesis, the segregating F_3 lines -- those with one to ten smutted plants -- were expected to contain, on the average, a ratio of one homozygous-resistant plant to two heterozygous to one homozygous-susceptible plant; that is, 7.5 : 15 : 7.5 in lines of 30 plants. If resistance had been dominant the heterozygous plants in the segregating lines would have been near-immune, and



DISTRIBUTION OF SMUTTED PLANTS IN HYBRID LINES

Figure 1



DISTRIBUTION OF SMUTTED PLANTS IN REDMAN SELECTION LINES

Figure 2

the homozygous-susceptible plants would have been the only ones that were smutted. In this case it should have been possible to predict the amount of loose smut infection in the segregating lines from that found in the susceptible lines -- those with more than eleven smutted plants. In these susceptible lines the average was 18.3 smutted plants or 61.0 per cent infection.* In the segregating lines it was therefore expected that 61.0 per cent of the 7.5 homozygous-susceptible plants would be smutted. This amounted to 15.4 per cent or 4.6 plants per line. The amount of infection actually exhibited by the lines classed as segregating was 14.4 per cent or 4.3 plants per line.

The close agreement obtained seemed to indicate that the Thatcher resistance actually was dominant. However, the problem was complicated by the presence of the moderate resistance of Redman Selection in the hybrid material. It is possible that the major gene of Thatcher was only partially dominant and that the addition of some or all of the Redman Selection resistance depressed the degree of infection obtained.

No conclusion was reached as to the mode of inheritance of the moderate resistance of Redman Selection.

Embryo Examinations

All of the Redman Selection and Thatcher lines tested by the Embryo Examination Method had at least one embryo containing loose smut

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* Percentages are weighted for the slight variations in size of lines.

hyphae. No difference was detected between the amounts of mycelium present in the two parents. A photomicrograph of the hyphae in one Thatcher embryo is shown in Figure 3. As Thatcher and Redman Selection could not be distinguished by this method, it was not used on the F_3 lines.

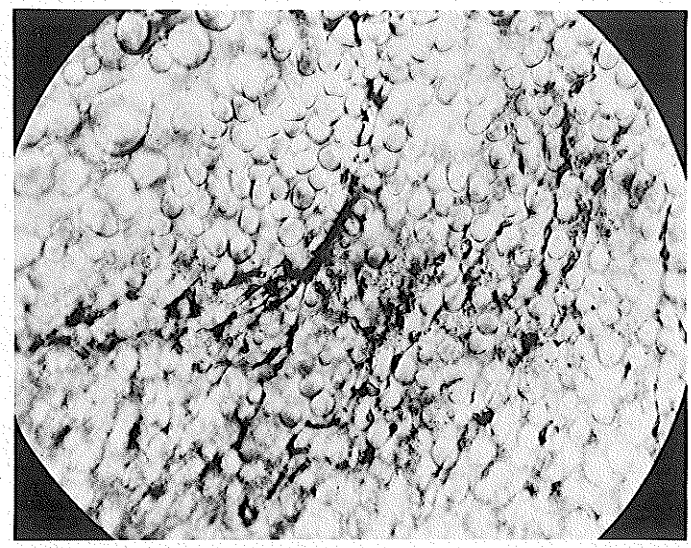
Seedling Examinations

A detailed classification of the results of the Seedling Examinations is given in Table IV. The classification of these same lines by the Mature Plant Examination Method is also given, in order that the results of the two methods can be readily compared.

The Redman Selection lines were all infected and were all classed as susceptible, in perfect agreement with the Mature Plant Examination Method. Five of the Thatcher lines were classed as resistant, and one as susceptible. This last disagrees with the results of the Mature Plant Examination. In spite of this slight disagreement the results show that Redman Selection and Thatcher can be readily distinguished by this method.

The results obtained by the two methods for the Redman Selection x Thatcher F_3 lines agreed for only 79.6 per cent of the lines. A summarized comparison of these results is shown in Table V. The Chi-square test of independence and association indicated a definite association between the results obtained by the two methods ($P<.01$).

As a further indication of this association, the correlation between the proportion of seedlings classified as smutted in each F_3



A PHOTOMICROGRAPH OF PART OF AN EMBRYO OF THATCHER WHEAT SHOWING DARKLY STAINED LOOSE SMUT HYPHAE
(x 250, approx.)

Figure 3

Table IV

COMPARISON OF RESULTS FROM SEEDLING AND FROM MATURE PLANT EXAMINATIONS

Plot & Line Number	Seedling Examination: Number Tested	No. of Seedlings With Infection of: 0	1	2	3	4	5	6	7	8	Resist. or Susc.	Mature Plant Exam.: Number of Smutted Plants	Resist. or Susc.
Redman Selection Lines													
3- 1	10	3	2	1	1	1	2	.	.	.	S	5	S
3	8	6	.	.	1	1	.	.	.	.	S	8	S
5	10	3	2	2	2	1	.	.	.	.	S	11	S
6	10	4	.	4	1	1	.	.	.	.	S	6	S
4- 2	10	1	.	3	2	1	3	.	.	.	S	6	S
4	6	5	.	.	1	.	.	.	.	.	S	2	S
5- 2	10	6	1	.	1	1	1	.	.	.	S	-	-
5	10	6	.	1	2	.	1	.	.	.	S	9	S
Thatcher Lines													
8- 1	10	10	.	.	.	.	.	.	.	.	R	0	R
3	10	8	1	1	.	.	.	.	.	.	S	0	R
9- 1	8	8	.	.	.	.	.	.	.	.	R	0	R
2	6	6	.	.	.	.	.	.	.	.	R	0	R
8	6	6	.	.	.	.	.	.	.	.	R	0	R
10- 1	8	7	1	.	.	.	.	.	.	.	R	0	R
Redman Selection x Thatcher F_3 Lines													
1- 3	10	9	1	.	.	.	.	.	.	.	R	2	S
4	6	6	.	.	.	.	.	.	.	.	R	0	R
5	10	3	.	.	6	1	.	.	.	.	S	3	S
6	10	10	.	.	.	.	.	.	.	.	R	0	R
8	8	7	.	1	.	.	.	.	.	.	S	0	R
9	10	9	.	.	1	.	.	.	.	.	S	4	S
11	7	6	.	1	.	.	.	.	.	.	S	5	S
13	10	7	1	1	1	.	.	.	.	.	S	15	S
17	10	6	1	1	2	.	.	.	.	.	S	2	S
18	10	9	.	.	.	1	.	.	.	.	S	2	S
19	10	9	1	.	.	.	.	.	.	.	R	3	S
21	10	10	.	.	.	.	.	.	.	.	R	0	R

(Concluded on next page)

Table IV -- Concluded

Plot & Line Number	Seedling Examination: Number Tested	No. of Seedlings With Infection of: 0	1	2	3	4	5	6	7	8	Resist. or Susc.	Mature Plant Exam.: Number of Smutted Plants	Resist. or Susc.
Redman Selection x Thatcher F_3 Lines, concluded													
1-26	10	8	.	.	1	1	.	.	.	.	S	1	S
27	10	10	.	.	.	.	.	.	.	.	R	0	R
28	10	9	.	1	.	.	.	.	.	.	S	4	S
30	7	3	1	.	.	2	1	.	.	.	S	18	S
32	10	5	2	1	2	.	.	.	.	.	S	2	S
33	8	.	2	4	2	.	.	.	.	.	S	8	S
34	10	6	.	3	1	.	.	.	.	.	S	6	S
35	5	1	1	.	1	1	1	.	.	.	S	27	S
37	10	8	1	1	.	.	.	.	.	.	S	4	S
38	10	10	.	.	.	.	.	.	.	.	R	1	S
41	10	8	1	.	.	1	.	.	.	.	S	0	R
42	8	1	.	.	5	1	1	.	.	.	S	27	S
43	10	3	.	.	2	1	2	2	.	.	S	3	S
2- 2	10	7	1	1	.	.	1	.	.	.	S	9	S
4	10	10	.	.	.	.	.	.	.	.	R	0	R
5	8	4	2	1	1	.	.	.	.	.	S	0	R
10	10	5	5	.	.	.	.	.	.	.	R	4	S
15	9	6	.	2	.	.	.	1	.	.	S	7	S
22	8	1	.	.	1	.	2	1	2	1	S	18	S
23	4	1	.	.	1	.	2	.	.	.	S	4	S
24	7	4	2	1	.	.	.	.	.	.	S	0	R
25	9	5	.	.	.	1	1	1	1	.	S	20	S
27	10	7	.	.	1	2	.	.	.	.	S	9	S
30	10	6	2	1	1	.	.	.	.	.	S	6	S
34	7	2	.	1	2	2	.	.	.	.	S	0	R
36	10	1	.	1	3	4	1	.	.	.	S	27	S
40	7	4	1	1	.	.	1	.	.	.	S	4	S
41	7	.	.	1	2	1	1	2	.	.	S	6	S
45	7	.	1	.	3	2	1	.	.	.	S	7	S
47	10	8	1	1	.	.	.	.	.	.	S	0	R
50	4	2	.	1	1	.	.	.	.	.	S	5	R
52	10	4	1	1	2	1	1	.	.	.	S	3	S
53	8	6	2	.	.	.	.	.	.	.	R	0	R
55	10	4	.	2	2	1	1	.	.	.	S	12	S
56	8	4	1	1	1	1	.	.	.	.	S	3	S
57	10	10	.	.	.	.	.	.	.	.	R	0	R
60	10	6	.	3	1	.	.	.	.	.	S	14	S

Table V

NUMBER OF LINES IN EACH CLASSIFICATION BY SEEDLING AND BY MATURE PLANT EXAMINATIONS

		Seedling Examinations	
		Resist.	Susceptible
Mature Plant Examinations	Resist.	7	6
	Susc.	4	32

line, and the proportion of smutted plants found in the same line by growing the plants to maturity was .572, which was significant at the one per cent point.

These results showed that the Seedling Examination Method gave an indication of the loose smut reaction and, if susceptible, of the degree of infection. This method was considered a promising one for selecting resistant varieties or lines in a comparatively short time. However, in its present state of refinement, the technique is hardly reliable enough to warrant its use in genetic studies.

SUMMARY AND CONCLUSIONS

A number of plants of Redman Selection, Thatcher, and the F_2 of a cross between these two varieties were artificially inoculated with Ustilago tritici Physiologic Race 1. The resulting lines were tested for loose smut reaction by several methods.

The mature plant examination method consisted of growing the lines to maturity to determine the number of smutted plants in each. Thatcher proved to be near-immune, while Redman Selection was moderately resistant. The segregation observed in the F_3 lines of the cross indicated that the near-immunity of Thatcher was controlled by a single gene, which was probably dominant.

In the embryo examination method, embryos of Thatcher and Redman Selection were treated and inspected microscopically for loose smut hyphae. The loose smut reaction of these varieties could not be determined by this method and consequently it was not used on the F_3 lines.

The seedling examination method involved growing the lines to the two-leaf stage and then, after a special staining procedure, examining the tillering node microscopically for loose smut hyphae. Redman Selection and Thatcher could be readily identified by their loose smut reaction, but the classification of the F_3 lines was only 79.6 per cent correct, presuming that growing the lines to maturity gave their correct reactions. However, on the whole, this method gave an indication of the loose smut reaction and the degree of in-

fection. It was considered a promising one for selecting resistant lines or varieties in a comparatively short time, but did not seem reliable enough to use in genetic studies.

ACKNOWLEDGMENTS

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APPENDIX

A METHOD OF EXAMINING EMBRYOS OF WHEAT AND BARLEY FOR LOOSE SMUT INFECTION

by W. POPP

1. Prepare the following solutions :

   A. Potassium hydroxide . . . . . 10 gm.
      Water . . . . . 90 cc.

   B. Lactic acid . . . . . 12.5 cc.
      Phenol . . . . . 12.5 cc.
      Glycerine . . . . . 12.5 cc.
      Water . . . . . 62.5 cc.
      Poirrier's Blue (Cotton Blue) . . 0.005 gm.

   C. Glycerine . . . . . 1 part
      Water . . . . . 2 parts

2. Cut kernels in half at right angles to the crease and macerate the portions containing the embryo with a solution of potassium hydroxide (Solution A). About one hour is required for macerating the seed if the solution is kept at the boiling point, and about twelve to sixteen hours if the solution is kept at room temperature.

3. After the contents of the endosperm become a soft jelly-like mass, pour contents of dish into a watch glass. Place the embryos in a small beaker containing a solution of potassium hydroxide. Embryos remaining within the seed coat may be forced out by applying slight pressure to seed coat.

4. Boil the embryos for about two minutes in the potassium hydroxide solution to remove any foreign matter that may be adhering to them.

5. Drain off the potassium hydroxide solution and boil the embryos in water for about two minutes.

6. Drain off the wash water and boil the embryos in the stain (Solution B) for about three and one-half to four minutes.

7. Pour contents of beaker into watch glass. Place embryos on microscope slide, add mounting medium (Solution C), and cover embryos with a cover slip. To flatten out embryos apply slight pressure to cover slip.

8. Examine embryos under microscope in strong transmitted light.