THE UNIVERSITY OF MANITOBA

OCCURRENCE OF PHYSIOLOGIC RACES OF LOOSE SMUT OF WHEAT, *Ustilago tritici* (Pers.) Rostr., IN BRAZIL, SOURCES OF RESISTANCE AND INHERITANCE OF RESISTANCE TO RACE T 2 IN THE INTERVARIETAL CROSS KENYA 340 Y.4.A.1. x IAS 52

by

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ABSTRACT

OCCURRENCE OF PHYSIOLOGIC RACES OF LOOSE SMUT OF WHEAT, *Ustilago tritici* (Pers.) Rostr., IN BRAZIL, SOURCES OF RESISTANCE AND INHERITANCE OF RESISTANCE TO RACE T 2 IN THE INTERVARIETAL CROSS KENYA 340 Y.4.A.1. x IAS 52

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The variability in Brazil of loose smut of wheat, *Ustilago tritici* (Pers.) Rostr., was studied, based on the reaction of a set of differential cultivars. Twelve physiologic races were identified amongst the 50 collections of spores that were tested. Five of the races would be classified as being similar to Canadian race T 2, but further differentiation was possible by using three supplemental differentials. Two races gave a reaction identical to race T 8 with the standard set of differentials, but they too could be further differentiated on the supplemental differentials. None of the five remaining races resembles any Canadian race previously identified. Four of them are virulent on the durum wheat Pentad and on several cultivars of common wheat in the set of differentials. This appears to invalidate claims that *formae speciales* of *U. tritici* exist on common and on durum wheat.

A collection of 68 cultivars of diverse origin has been established that are resistant to races of loose smut from Brazil, Canada and other countries. Resistance was

confirmed of the cultivars Sinvalocho M.A., Maria Escobar and their derivatives, whose resistance had been reported earlier.

The inheritance of resistance to race T 2 of loose smut was studied in the intervarietal cross Kenya 340 Y.4.A.1. x IAS 52. It was found that the incompatible reaction to race T 2 of the resistant parent Kenya 340 Y.4.A.1. was dominant. Based on the performance of F3 progenies, it appears that a single gene is responsible for the resistance displayed by Kenya 340 Y.4.A.1., but that this gene is independent from those causing the incompatible reaction.

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CHAPTER 1

INTRODUCTION

Loose smut of wheat, caused by the fungus Ustilago tritici (Pers.) Rostr., is one of the diseases of wheat found in Brazil, and all cultivars recommended for cultivation in that country are to some degree susceptible. Data on overall annual losses to this disease are lacking but, in individual fields, levels of infection up to 10% have been recorded causing an equally high loss in yield. Although seed treatments in the form of modern fungicides are available today, breeding for resistance remains the most economical form to prevent the disease.

Before a programme of breeding for resistance to loose smut can be initiated in Brazil, it is essential to obtain information on certain aspects of the disease. First, since loose smut can be subdivided into physiological races that differ in virulence, it should be determined whether such races occur in Brazil and, if so, their characteristics should be established. Secondly, sources of resistance to these races had to be found. And, thirdly, the mode of inheritance of resistance to one of the prevalent Brazilian races, located in a suitable donor, should be studied using one of the presently grown Brazilian cultivars as the susceptible parent. Accordingly, the three phases of the present study followed these objectives.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Physiologic races of loose smut of wheat

The first indication that physiologic races of loose smut of wheat occur appears to have come from Piekenbrock (1927). By inoculating a number of cultivars of wheat with several collections of spores, he was able to identify two physiologic forms. Piekenbrock's work was continued by Grevel (1930) who studied 19 collections of loose smut from Germany, and 29 from countries other than Germany. These collections yielded four physiologic races, three from the German collections, while the fourth race originated from Turkey.

Since then, physiologic races of loose smut of wheat have been found in many countries, e.g. in Bulgaria (Mitov 1958), France (Simon and Croisier 1959), Great Britain (Batts 1955; Doling and Hervey-Murray 1966), The Netherlands (Oort 1944), Poland (Heinrich 1973), Romania (Radulescu 1935), USSR (Krivchenko 1970), India (Dastur 1946), China (Wang 1942), New Zealand (Cunningham 1940), South Africa (Gorter 1964), USA (Bever 1947, 1953).

In Canada, Hanna (1937) described four physiologic races of loose smut of wheat which he identified in Winnipeg from three collections of spores originally made in 1929 from the cultivars Reward, Kota and Mindum. Later, Cherewick (1953) described the ten physiologic races that had been differentiated in Canada up to that time by using, with few

modifications, the group of cultivars selected by Oort (1944) in The Netherlands. At present, the identification of races in Canada is still based on the group of differential cultivars used by Cherewick (1953) although four additional cultivars have been added to identify races not differentiated on the cultivars used by Cherewick.

Unfortunately, the absence of a standard group of differential cultivars, and the different methods of classification of the cultivars regarding reaction to races of loose smut, do not permit a comparison of the results obtained by workers in different countries. Therefore, in the present study, the pattern of virulence of physiologic races from Brazil can only be compared with that of races under study in Canada.

Very little is known about pathogenic specialization of loose smut in South America. In Brazil, Silva (1951) noted possible differences in pathogenicity occurring within the population of loose smut in the State of Rio Grande do Sul. He based this on the fact that the cultivar Planalto was resistant when artificially inoculated with samples from one region of that State, whereas the same cultivar was naturally infected in another region. Mascarenhas and Silva (1954) working with three samples of spores collected in the State of Rio Grande do Sul, and one from the State of Parana, found differences in pathogenicity between the samples when a collection of commercial cultivars was tested in Pelotas, Brazil, during the period 1950-54. Although these two

studies suggested the presence of physiologic races of loose smut of wheat in Brazil, they gave no information as to their number, nor to their variability in pathogenicity.

In all of the above-mentioned studies, the classification of a race was based on one of two reactions of the host-parasite system: spore-formation, or no spore-formation. However, only Oort (1944) has pointed out that a failure to produce spores, which was normally equated with resistance, could be caused not only by physiologic resistance in the proper sense, but also by hypersensitivity of the host. From observations made and experiments carried out, Oort concluded that two different principles were involved in the reaction of plant and parasite. The first was a principle of susceptibility or non-susceptibility; it determines whether the plant will be resistant (no symptoms) or susceptible (shows symptoms). The second principle was that of hypersensitivity or non-hypersensitivity and this determines whether the susceptible plant will show abnormal symptoms, or normal ones with smutted ears. The abnormal symptoms, or hypersensitive reaction of wheat to loose smut, were described by Oort (1944) as follows:

> A strong growth inhibition, which is shown by a shortening of the first three leaves, is the most striking symptom. It may be accompanied by chlorotic stripes and spots and by malformation causing a curling of the leaves. These symptoms appear only clearly when the inoculated seed is sown in the greenhouse under favourable conditions. Many plants showing these symptoms die

> in the two- or three-leaves stage. The survivors recover slowly either by the main axis coming to new growth, or, when this dies, by the developing of side-shoots. The plants which recover remain in all dimensions much smaller than normal plants and are - with a few exceptions - healthy, i.e. they show no smut.

Since no spores are formed on these hypersensitive plants they were termed "field resistant" by Oort. Later, Oort (1963) worked out a genetical model to fit his hypothesis. Two pairs of independent factors controlled the reaction of the host to a given race: one pair determined resistance or susceptibility, the other pair determined whether the plant, if susceptible, would react with hypersensitivity or not. Correspondingly, the parasite also had to carry two pairs of independent factors: one pair of genes determined avirulence or virulence; the other pair determined whether hypersensitivity could be overcome or not.

As mentioned above, the phenomenon of hypersensitivity observed by Oort was not used by other authors as a characteristic to distinguish physiologic races of loose smut of wheat. On the other hand, Kiraly and Lelley (1957) studying six winter wheat cultivars aritificially inoculated with loose smut, confirmed the presence of hypersensitivity in some wheat-smut combinations. They examined inoculated wheat plants for the presence of smut in the culms after heading and while they found the fungus in one half of the depressed plants, it never reached the highest node. In the culms of plants which did not show a hypersensitive reaction, the fungus could not be detected. They concluded

that hypersensitivity of the host either restricts spread of the parasite so that it cannot reach the organ where it could insure its reproduction, or it totally eliminates the fungus and the host recovers from the disease. Mantle (1961), after anatomical examination of plants with abnormal reaction to loose smut of wheat, showed that the term "hypersensitivity" was not the proper one to use for the observed phenomenon. A hypersensitive reaction to fungal invasion is usually defined as being a very localized necrosis, thereby preventing further growth of the parasite. In the abnormal reaction of wheat to loose smut, however, the whole plant is affected, and growth of the parasite is retarded. Mantle (1961) used the more general "incompatibility", and this term will be used in the present study, although even it may no longer be acceptable today. A further clarification concerns the terms "infection" or "infected plants". In the following they are used in the restricted sense of being synonymous with "sporulation" or "plants that show spore-formation"; they do not include plants that show incompatibility which undoubtedly had been infected also.

2.2. Sources of resistance to loose smut

Fischer and Holton (1957) stated that wheat cultivars may react with different degrees of resistance or susceptibility to loose smut. Such reactions are common amongst many different cultivars in widely separated geographical areas. In some cases, resistant and susceptible reactions seemed to be related to the species or type-group of cultivars, while similar relationships could not be established in other cases. After a review of early works the authors said:

> In sum total, the information on varietal resistance has established the availability of high resistance to loose smut in a limited number of commercial varieties and in a wide range of breeding stock. Thus, the development of new, agronomically desirable types with high resistance to loose smut is assured through appropriate breeding procedures based on the nature of inheritance of resistance factors.

For a given area, such a breeding program will usually be based on a screening of the reaction of adapted local cultivars and foreign introductions to smut by means of artificial inoculation with races or field collections from that area. In South America, only a few such studies have been done. Silva (1951) tested 127 wheat cultivars in Brazil with one field collection of loose smut. He found the following to be resistant: Ardito, Bandeirante, Barbela 2711, Bonaerense, Celebration, Charuto, F.P.I. 104137 x 41-116, Florence, Garnet, General Vargas, Hope, (Hope x Med. x A.M.) x Gaza, Joana, Kendee, Klein Acero,

Klein Orgullo, Klein Universal, M-2-38, M-18-38, Maia 9994, Mindum, Minn. 2676, Nordeste, Newthatch, Pilot, Planalto, Purplestraw, Pusa 52, Rival M-4-38, Renascimiento and Santa Marta (37/46). Mascarenhas and Silva (1954) tested 47 cultivars with one field collection of loose smut. The cultivars they found to be resistant were among those found resistant in the earliest test (Silva 1951). A cultivar tested for the first time was Sinvalocho M.A., and it was immune.

In Argentina, Cenoz (1952) inoculated a collection of 441 cultivars at Castelar, Buenos Aires, with two field collections of smut from common wheats. His purpose was to determine the reaction of all cultivars grown in Argentina at that time, as well as of some selected foreign cultivars. Sources of resistance would thus be determined which could be used in Argentina and neighboring countries. As a result of the tests performed between 1945-49, the cultivar Sinvalocho M.A. was the only Argentine cultivar that was determined to be immune to loose smut. Other local cultivars that showed high resistance were: 38 M.A., Buck Quequen, Klein Aniversario, Klein Cometa, Klein Exito, Klein Orgullo, Klein Otto Wulff, Klein Progreso and Reliance Sel. Klein. Almost all of these cultivars have one of the following in their pedigree: Marquis, Reliance, Klein 49a, or Chino 466. Among the foreign cultivars the following were immune: Apex, Axminster, Carina, Chino 466, Chul, Dixon, Fultz, Giza 121,

Heines Kolben, Kendee, Maria Escobar, Newthatch, Rapier, Redhart Strain 5 and Riosulino. All durum wheat cultivars tested, including Mindum and Pentad, were either immune or highly resistant.

Later, in Argentina, Frecha (1967) reported on tests he had conducted from 1963-66. In these, 80 cultivars and lines of wheat including Argentine cultivars and interesting breeding material were artificially inoculated with a mixture of an unknown number of field collections of loose smut. Among the Argentine cultivars, El Gaucho F.A. was found to be immune, whereas Agrolit Vagliano, Pergamino Gaboto M.A.G. and Olaeta Artillero were highly resistant. Among the breeding material the following were immune: Barleta Benvenuto, (Chin. x Aeg. umbellulata) x Thatcher,[6] Chino 466, Dundee x Kenya B.C.4/1.2.1.1., Egypt Na 101, Magnif MG, Maria Escobar, Massaux 5, Sinvalocho M.A. and 38 M.A. With very few exceptions, the cultivars found to be immune or resistant by Frecha were those found to be immune or resistant in the tests reported on by Cenoz (1952). The exceptions were the cultivars Klein Orgullo and Klein Aniversario, reported to be resistant by Cenoz, but susceptible in the tests of Frecha (1968). Probably one of the collections used by Frecha carried virulence on these cultivars, while the virulence of the rest of his inoculum was identical to the virulence of the inoculum used by Cenoz (1952).

A deficiency in these screenings of cultivars for resistance to loose smut in Brazil and Argentine, was the low number of field collections used in the inocula. Also, without knowledge of the pathogenic variation within these few collections and within the population as a whole, the cultivars were likely to have been exposed to only a small part of the virulence present in those countries.

2.3. Inheritance of resistance to loose smut

According to Kilduff (1933) the first report on inheritance of resistance to loose smut of wheat was made by Olson et al. (1920). A review of their work by Matsuura (1929) states: "There are several genes involved for resistance to smut caused by Ustilago tritici in different varieties."

Piekenbrock (1927) working with crosses of a highly resistant cultivar and two susceptible spring wheats found that segregation in the F2 and F3 generations indicated that immunity was inherited recessively. Grevel (1930) confirmed Piekenbrock's conclusion.

Kilduff (1933) studied the F3 and F4 generations of the crosses Kota x Red Bobs and Kota x Garnet. Although Kota was relatively susceptible, Garnet resistant, and Red Bobs immune to the collection of loose smut used, Kilduff could not fit the levels of infection in the segregating material into any ratio that would be indicative of the action of either one or a few genes. He suggested that the resistance of the parental cultivars might have a basis other than physiological.

Rudorf and von Rosenstiel (1934) indicated that the resistance to loose smut of the cultivar 38 M.A. probably depended on three recessive factors. They concluded this from the reaction of the F3 generation of a cross with the susceptible cultivar San Martin.

Tingey and Tolman (1934) made the crosses Hope x Federation, Preston x 01-24 and Hope x Dicklow. They concluded from the segregating generations that at least three factors were involved in the inheritance of resistance to loose smut, that resistance was dominant, and, though dominance was incomplete, the factors had a cumulative effect.

Heyne and Hansing (1955) observed that resistance of Kawvale to race 11 of U. tritici was dominant over the susceptibility of Clarkan and dependent upon at least two factors.

Based on the segregation observed in F3 lines of the cross Thatcher x Redman Selection, Campbell (1948) concluded that the near immunity of Thatcher to the Canadian race 1 of loose smut was controlled by a single gene, which was probably dominant.

Mathur and Kohli (1963) studied the F1, F2 and F3 of a cross between the resistant cultivar N.P. 824 and the susceptible Rio Negro and concluded that resistance was dominant and monogenically controlled.

Agrawal et al. (1963) studied the inheritance of resistance to loose smut in the cross N.P. 775 x N.P. 798. From the reaction of the F1, F2 and F3 they concluded that the resistance of N.P. 798 was conditioned by two pairs of dominant duplicate genes.

Agrawal and Jain (1965), after observing the reaction of the F1, F2 and F3, reported that loose smut resistance of N.P. 790 in a cross with the highly susceptible N.P. 775 was governed by a single dominant factor.

Shestakova and Vjushkov (1974) pointed out that in the material they studied, resistance was determined by a small number of genes with strong additive and weak dominant effects. Accordingly, immunity to race 16 of the cultivar "Bezenchukskaya 98" was determined by 3 genes; the high resistance of "Saratovskaya 36" by 2 genes, and moderate resistance of "Saratovskaya 29" by a single gene.

CHAPTER 3

MATERIALS AND METHODS

3.1. Physiologic races of loose smut of wheat in Brazil

Fifty samples of loose smut were used to study the variability of the loose smut fungus in Brazil. In order to reduce the possibility of working with a mixture of races each sample consisted of only one smutted head. Some of the samples were collected at Winnipeg on cultivars originally inoculated with mixture A or B. Mixture A was made up from spores collected on different cultivars in different areas of Brazil; mixture B was formed only from spores collected on the naturally infected Brazilian cultivar IAS 52. Other samples of spores were received from Brazil in 1973 and these had been collected on naturally infected cultivars (identified by N in table below). Table 1 gives details of the samples tested.

The following differential cultivars were used to identify the virulence pattern of the Brazilian samples of loose smut: (1) Mindum, (2) Renfrew, (3) Florence x Aurore, (4) Kota, (5) Little Club, (6) (van Hoek), (7) Reward, (8) Carma, (9) Kearney, (10) Red Bobs, (11) Pentad, (12) Thatcher x Regent, (13) P.I. 298 554, (14) Sonop and (15) H44 x Marquis.

The above set of cultivars is used at Agriculture Canada, Research Station, Winnipeg, to identify physiologic races of loose smut of wheat. However, this set had to be supplemented by the cultivar Klein 40, because in 1973 it

TABLE 1. Origin of samples used in the study of physiologic races of loose smut from Brazil.

Sample	Collected on cultivar	at	after inoculation with
1	IAS 52	Winnipeg	A
2	IAS 52	Winnipeg	A
3	IAS 52	Winnipeg	A
4	IAS 52	Winnipeg	A
5	IAS 52	Winnipeg	A
6	IAS 52	Winnipeg	A
7	IAS 52	Winnipeg	A
8	IAS 52	Winnipeg	A
9	IAS 52	Winnipeg	A
10	IAS 52	Winnipeg	A
11	L. 8220-54	Winnipeg	A
12	PF 72574	Passo Fundo	N
13	Klein 40	Winnipeg	A
14	IAS 52	Winnipeg	B
15	Line 1262-26	Winnipeg	B
16	PF 72576	Passo Fundo	N
17	Klein 40	Winnipeg	B
18	Kenya 4121	Winnipeg	B
19	Parana 62/1845	Winnipeg	A
20	Parana 62/1845	Winnipeg	B
21	IAS 50-Alvorada	Vacaria	N
22	IAS 50-Alvorada	Passo Fundo	N
23	IAS 51-Albatroz	Passo Fundo	N

TABLE 1. Origin of samples used in the study of physiologic races of loose smut from Brazil. (Cont.)

Sample	Collected on cultivar	at	after inoculation with
24	IAS 52	Vacaria	N
25	IAS 52	Passo Fundo	N
26	IAS 52	Passo Fundo	N
27	IAS 56	Passo Fundo	N
28	IAS 60	Vacaria	N
29	IAS 60	Passo Fundo	N
30	IAS 62	Vacaria	N
31	Cinquentenário	Vacaria	N
32	Encruzilhada	Passo Fundo	N
33	IAS 51-Albatroz	Passo Fundo	N
34	Pat 16	Passo Fundo	N
35	Pat 49	Passo Fundo	N
36	PF 70338	Passo Fundo	N
37	PF 70412	Passo Fundo	N
38	PF 70440	Passo Fundo	N
39	PF 70592	Passo Fundo	N
40	PF 70358	Passo Fundo	N
41	PF 7110	Vacaria	N
42	PF 7159	Passo Fundo	N
43	PF 7197	Passo Fundo	N
44	PF 71108	Passo Fundo	N
45	PF 71111	Passo Fundo	N

TABLE 1. Origin of samples used in the study of physiologic races of loose smut from Brazil. (Cont.)

Sample	Collected on cultivar	at	after inoculation with
46	PF 72390	Passo Fundo	N
47	PF 72121	Passo Fundo	N
48	PF 72199	Passo Fundo	N
49	PF 72202	Passo Fundo	N
50	PF 72238	Passo Fundo	N

was found that the previously resistant Klein 40 was susceptible to race T 9, which originated in Czechoslovakia (Nielsen, unpublished results), as well as to samples of smut from Brazil. Two single-plant lines, SPL 1 and SPL 2, from the Brazilian cultivar IAS 52 were also included as supplemental differentials. These two lines were selected from a set of five single-plant lines of IAS 52 that served to determine which race should be used in the study on inheritance of resistance. When inoculated with Canadian races T 1 to T 10 it was found that the first line (SPL 1) differed in its reaction from the other four (SPL 2 among them). SPL 1 showed low susceptibility to T2 and high susceptibility to T 8, but lines 2 to 5 were highly susceptible to T 2 only.

The differential cultivars were grown in pots in a greenhouse. At mid-anthesis, three heads of each cultivar were inoculated (using hypodermic needle and syringe) with a suspension of spores from each of the 50 samples of spores. Inoculated seed was grown in the field and percent infection established from an estimate of healthy and diseased heads.

Samples that gave identical patterns of virulence were grouped, a typical sample was selected from each group, and a single infected head collected from one of the differentials. Spores from this head were used to reinoculate a second set of differentials to repeat the test. However, the test was not repeated with three groups in which the