Evaluation of Oats for Resistance to Loose Smut

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ABSTRACT

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The percentage of loose smut (*Ustilago avenae*) infected plants in oat (*Avena sativa*) cultivars and breeding lines was determined after kernels were inoculated in a vacuum with water suspensions of teliospores. Fifty-two oat cultivars were evaluated between 1978 and 1990; 14 were resistant, six were moderately resistant, and 32 were susceptible. Many advanced breeding lines were also resistant. Factors that might affect the incidence of smut infection in evaluation trials were studied with cultivars Moore (moderately resistant), Ogle (susceptible), and Starter (resistant). One to three exposures in the vacuum during the inoculation procedure did not greatly affect the incidence of loose smut in the cultivars. As inoculum concentration decreased, the incidence of loose smut decreased, but incidence was not generally affected by storing inoculated seed for 6 wk before planting. In these tests, Starter consistently had the least infection, and Ogle the most; Moore was intermediate. Removal of the lemma and the palea prior to inoculation increased the incidence of smut 10–20%, depending on the cultivar.

Loose smut, caused by *Ustilago avenae* (Pers.) Rostr., was severe during 1972 in the oat (*Avena sativa* L.) cultivar Froker, which was the most widely grown cultivar in Minnesota. Until 1977, 50–80% incidence of loose smut was common in many fields throughout the state. Since 1977, loose smut has been controlled in Minnesota by the widespread use of resistant cultivars.

The objectives of this study were 1) to document procedures used in Minnesota for evaluating loose smut reactions in oat; 2) to evaluate some of the factors that might influence the success of these procedures; and 3) to evaluate oat cultivars, especially those developed at the University of Minnesota (4–9), for loose smut reaction.

MATERIALS AND METHODS

Procedures for inoculation and disease evaluation. From 5 to 10 g of kernels of each oat cultivar or breeding line was placed in a test tube (2.54 × 15.24 cm). The test tubes were then placed in wire baskets (30–40 tubes per basket) and filled with inoculum. The inoculum was a mixture containing teliospores of *U. avenae*: 12.5 g of teliospores per liter of water containing 10 ml of Tween 20. The test tubes were plugged with metal gauze to prevent the loss of kernels during the inoculation process, then placed in a chamber for vacuum infiltration of the teliospores (2,3,11). A vacuum of about 127 mm of mercury (12,868 kPa) was drawn for 3 min and then released. This vacuum procedure was repeated three times in rapid succession. After inoculation, the kernels were placed on a paper towel and allowed to dry overnight, then put in envelopes for storage before planting.

The teliospores were collected the year before the tests and held at 5 C until used. Each year the teliospores were collected from different cultivars and breeding lines in the oat loose smut nursery of the University of Minnesota, and occasionally from farm fields in various parts of Minnesota. Originally, teliospores were collected from Froker and from unidentified cultivars in different parts of Minnesota.

Planting each year was during the last week of April or the first week of May at Rosemount, Minnesota. Five to 10 grams of kernels per cultivar or line was planted with a plot seeder set to drop kernels 6 cm deep in rows 3.1 m long and 30 cm apart. Weeds were controlled manually and with the herbicides bromoxynil (ME 4 Brominal), propachlor (Ramrod), and dicamba (Banvel) applied preemergence, and with propanil (Stampede) applied 3 wk after planting at recommended rates. Plots were sprinkler irrigated when necessary to maintain vigorous plant growth.

About 1 wk after the inflorescence had emerged from the boot, the incidence of loose smut was determined. In each row, the percentage of plants infected was estimated as 0 (no infected plants observed), 1% (1–5 infected plants), 5% (determined by counting plants), or 10% or higher in units of ten.

Evaluation of infection factors. *Experiment I.* To test the effects of inoculum concentration and the number of exposures in a vacuum, kernels of Moore, Ogle, and Starter were inoculated with 3.0–17.0 grams of teliospores per liter of water containing Tween 20, and exposed 1–3 times in a vacuum for 3 min each time. Kernels were planted immediately after inoculation at St. Paul, Minnesota. The experiment was a randomized complete block with four replicates. The treatments were arranged as a split-split plot with cultivars in the main plots, inoculum concentrations in the split plots, and vacuum treatments in the split-split plots. The experiment was run in 1989, 1990, and 1991; but only the data for 1989 are shown because each year's results were comparable to those of many previous trials.

Experiment II. To test the effect of storing inoculated kernels for different lengths of time before planting, kernels of Moore, Ogle, and Starter were inoculated in 1990 by the standard procedure (described earlier) and stored at room temperature in the laboratory. Two rows of each treatment were planted in the field at St. Paul immediately after inoculation and at weekly intervals thereafter for 5 wk. In 1991 kernels of Moore, Ogle, and Starter were inoculated by standard procedures and stored at room temperature for 5 wk. Each week, just prior to planting, another set of kernels of each cultivar was inoculated and planted adjacent to the stored kernels. Two rows of each treatment were planted.

Experiment III. To test the protection provided by the lemma and palea, these structures were removed or left intact on kernels of Ogle, Starter, and ND 820559 (moderately resistant). Moore (moderately resistant) was used instead of ND 820559 in a second trial. Each trial had three replicates of about 50 kernels of each cultivar and the lemma/palea treatments. Kernels were inoculated by the standard procedure and planted in the glasshouse in January 1989 and 1991. The experiment was a randomized block design arranged as a split plot with cultivars in main plots and the lemma and palea treatments in the split plots. The incidence of smut-infected plants was determined about 50 days after planting, when heading was complete.

Evaluation of cultivars. The number of cultivars tested each year varied with their availability and with our interest in their loose smut reactions. In addition, about 1,500 breeding lines in the F_5 or F_6 generation were evaluated each year. Most cultivars and breeding lines were

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planted as single rows each year, but check cultivars and advanced lines considered to be potential cultivars were planted several times annually.

Resistant cultivars were defined as those with mean loose smut incidence of 5% or less, and a maximum not exceeding 20%. Moderately resistant cultivars had mean smut incidences of 6–20%, with a maximum not exceeding 40%. Susceptible cultivars had mean incidences of 26% or more, with a maximum of 50% or more.

Year-to-year variation in the incidence of loose smut was estimated with the cultivars Hazel and Ogle (both susceptible) and Moore and Steel (both moderately resistant) between 1985 and 1989. In addition, year-to-year variation was estimated with cultivars Benson, Lyon, and Noble (all resistant) and Moore (moderately resistant) compared with Marathon or Ogle (both susceptible) in 1979 through 1984. In both sets of comparisons, each cultivar was represented in the nursery a minimum of three times and usually more than 10.

RESULTS

Evaluation of infection factors. As the inoculum concentrations decreased from 17.0 to 3.0 grams of teliospores per liter of water, the incidence of infection also decreased. Starter consistently had the least disease, and Ogle the most; Moore was intermediate (Table 1). The number of exposures in the vacuum procedure did not significantly affect the incidence of loose smut in the cultivars (*data not shown*). Interactions among the experimental variables were nonsignificant.

Storage of inoculated kernels for 0–5 wk before planting generally did not affect loose smut incidence (Table 2). Again, Starter had the lowest percentage of infected plants, and Ogle the highest; Moore was intermediate. This ranking also was consistent when inoculated kernels were stored 1–5 wk after inoculation and compared with freshly inoculated kernels. The incidence of loose smut was least in Starter, greatest in Ogle, and intermediate in Moore (Table 3).

Removal of the lemma and palea from kernels prior to inoculation significantly increased the incidence of infection in each cultivar (Table 4). Cultivars also differed in their reaction to loose smut; the incidence of infection was greatest in Ogle, intermediate in ND 820559 and Moore, and least in Starter. The interaction of cultivars × removal of the lemma and palea was not significant in both experiments.

Evaluation of cultivars. Loose smut reactions of 52 cultivars were evaluated between 1978 and 1990; 14 were resistant, six moderately resistant, and 32 susceptible (Table 5).

Year-to-year variation in the incidence of loose smut was evaluated with cultivars that had high or low incidence of infection. In tests between 1985 and 1989, the incidence of infection was about equal in Moore and Steele, and was significantly less than in Hazel and Ogle (Table 6). In tests of 1979 through 1984, the mean percentage of loose smut infected plants was low in Benson, Lyon, and Noble; intermediate in Moore; and high in the susceptible cultivars, Marathon and Ogle (Table 7). In both sets of comparisons, the effects of the year of testing were not significant.

DISCUSSION

The reactions of oat cultivars in North America to loose smut have been reported often in the last 70 yr; however, the most recent report was made 13 yr ago by Nielsen (3). Our list of resistant cultivars is not as long as that of Nielsen, but it includes sources of resistance that may be useful, especially against the current pathogen population in Minnesota.

The virulence/avirulence of the population of *U. avenae* used in this study

Table 1. Percentage of plants in three oat cultivars infected with *Ustilago avenae* after inoculation with varying concentrations of teliospores in 1989

	Infection percentages with five teliospore concentrations (g/L)[z]					
Cultivar	17.0	13.0	10.0	7.0	3.0	Mean
Moore	7.6	5.5	6.8	4.8	2.0	5.4 a
Ogle	45.0	31.7	38.3	30.8	20.9	33.3 b
Starter	1.0	1.0	1.0	1.0	1.0	1.0 c
Mean	17.9 a	12.7 b	15.4 ab	12.2 b	7.9 c	

[z] Means of cultivars and inoculum concentrations were significant ($P = 0.05$), but the cultivar × inoculum interaction was not significant. Means followed by the same letter were not significantly different according to Duncan's multiple range test. Similar studies were made in 1990 and 1991 with comparable results.

Table 2. Percentage[z] of plants in three oat cultivars infected with *Ustilago avenae* when kernels were inoculated and planted immediately (0) or after 1–5 wk of storage at room temperature

	Weeks between inoculation and planting					
Cultivar	0	1	2	3	4	5
Moore	30	20	20	20	30	30
Ogle	60	40	70	30	60	60
Starter	1	1	0	1	0	3

[z] Mean based on two rows per cultivar for each treatment.

Table 3. Percentage[z] of plants in three oat cultivars infected with *Ustilago avenae* (A) when kernels were inoculated and planted after 1–5 wk of storage at room temperature, and (B) when kernels were inoculated and planted immediately on each planting date

	Weeks after inoculation									
	1		2		3		4		5	
Cultivar	A	B	A	B	A	B	A	B	A	B
Moore	20	20	20	20	20	20	30	30	30	30
Ogle	40	50	70	60	60	70	60	60	60	60
Starter	1	3	1	2	1	1	0	0	1	1

[z] Mean based on two rows per cultivar for each treatment.

Table 4. Mean percentage of plants infected with *Ustilago avenae* in oat cultivars with lemma and palea removed or left intact

	Infected plants (%)[z]		
Cultivar	Intact	Removed	Mean
Experiment one			
Ogle	53.3	66.2	60.0 a
Starter	0.7	7.9	4.3 b
ND 820559	13.6	32.9	23.3 c
Mean	22.5 a	35.7 b	
Experiment two			
Ogle	53.2	69.1	61.2 a
Starter	6.0	27.0	16.5 b
Moore	34.5	41.5	38.0 c
Mean	31.2 a	45.8 b	

[z] Differences due to cultivar and removal of lemma and palea were significant ($P = 0.05$), but the interaction of cultivar × removal was not significant.

Table 5. Mean and range of the percentage of plants in each oat cultivar infected with *Ustilago avenae*: 1978–1990[z]

Cultivar	Years tested (no.)	Total tests (no.)	Loose smut	
			Mean (%)	Range (%)
Resistant				
Andrew *	3	3	1.7	0–05
Benson *	8	121	2.3	0–20
Don	7	85	3.6	0–10
Dumont	4	8	1.3	0–05
Fidler	7	9	0.0	...
Hytest	3	3	5.0	...
Lyon *	11	150	0.8	0–10
Noble	9	75	1.5	0–10
Porter	4	13	0.2	0–05
Premier *	3	66	0.1	0–1
Preston *	8	48	4.7	0–20
Proat *	4	12	2.1	0–10
Robert	3	3	3.3	0–10
Starter *	6	91	1.3	0–20
Moderately resistant				
Centenniel	2	4	15.1	5–20
Dal	11	24	10.1	1–40
Moore *	12	192	16.5	0–40
Settler	2	3	16.7	10–20
Steele	16	98	20.0	0–30
Trucker	1	2	20.0	10–30
Susceptible				
Bates	4	4	35.0	20–50
Chief	3	20	32.8	0–80
Clintland 64	5	6	26.8	5–80
Clintford	4	4	52.5	50–60
Froker	5	12	41.8	30–50
Garry	3	3	50.0	30–60
Goodland	4	15	35.3	0–50
Gopher	11	11	34.5	10–80
Hamilton	1	1	50.0	...
Haylander	3	3	40.0	20–50
Hazel	6	91	52.4	5–60
Horicon	2	3	65.0	20–80
Keley	1	1	40.0	...
Kelsey	3	4	40.0	20–70
Lance	1	2	43.0	20–70
Lancer	7	8	31.3	10–50
Lang	3	35	32.7	10–60
Larry	3	7	44.0	30–70
Marathon	3	12	47.0	40–60
Lodi	1	4	52.5	30–80
Marion	1	1	60.0	...
Newdak	1	2	45.0	30–60
Ogle	10	91	54.0	40–80
Orbit	3	3	37.0	5–60
Otana	4	4	32.5	5–50
Otee	5	16	42.5	40–60
Pennlo	1	1	60.0	...
Rodney	6	8	36.5	30–50
Sandy	3	3	53.3	50–60
Stout	7	33	40.9	20–80
Valley	3	24	32.9	10–70
Webster	7	8	48.8	10–60

[z] Inoculated by vacuum procedure and planted in the field at Rosemount, Minnesota. Asterisk indicates cultivars released by the University of Minnesota.

Table 6. Mean percentage of plants in oat cultivars inoculated with *Ustilago avenae* in tests from 1985 to 1989[z]

Cultivar	1985	1986	1987	1988	1989	Mean
Hazel	41	42	51	53	58	49.0 b
Moore	24	24	21	2	27	19.6 a
Ogle	53	49	58	77	55	58.4 b
Steele	26	27	21	39	19	26.4 a
Mean	36.0	35.5	37.7	42.7	39.8	

[z] Data are means of several observations for each cultivar each year: 18–22 observations per cultivar for 1985, 13–22 per cultivar for 1986, 4–19 per cultivar for 1987, 3–12 per cultivar for 1988, and 3–12 per cultivar for 1989. Differences due to cultivar were significant, but not those due to year of testing. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

differs from that of isolates used by Nielsen (3), Holton and Rodenhiser (2), and Hansing et al (1). Our population was obtained primarily from the cultivar Froker, and its virulence on smut differential cultivars was checked four times between 1979 and 1990. In the differential cultivars Anthony, Black Diamond, Black Mesdag, and Victory, the incidence of infection was 30–90%; in Camas, Markton, Monarch, and Navarro 0–10%; in Fulgam and Nicol 1–20%; in Gothland 2–30%; and in Clintland 64 and Victoria 10–30% (Table 8). We attempted to create a diversity of virulence in the inoculum by occasionally adding teliospores collected from unknown cultivars. However, data from the infection of the differential cultivars suggest that the Minnesota population of the pathogen consists of closely related isolates.

We placed cultivars and lines in resistance classes on the basis of observed incidence of loose smut after several tests had been made. The cultivars released by the University of Minnesota (4–9)—Andrew, Benson, Lyon, Moore, Premier, Preston, Proat, and Starter—were evaluated three to 192 times. These cultivars continue to be as resistant as when they were released. Over the years, they have either been smut free or shown only traces of disease in Minnesota production fields.

Cultivars should be tested as many times as possible to ensure that the correct reaction to loose smut has been observed. In susceptible cultivars, we have observed a variation of 0–80% infected plants, and in resistant cultivars 0–10%. A cultivar with a high incidence of infection in one or two trials probably should be classified as susceptible, because its potential has been shown. However, a cultivar with a single low incidence of infection cannot be considered resistant without further testing.

Many factors may influence the development of loose smut in oat, but those that affect the success of inoculation may be the most important. The lemma and palea are reported to help prevent the pathogen from contacting the embryo and the young seedlings, thereby reducing the incidence of infection (10,11). This was confirmed with cultivars Ogle (susceptible), Starter (resistant), and ND 820559 and Moore (moderately resistant). Though the lemma and palea provide some protection against infection, the vacuum inoculation method helps avoid large errors in evaluating resistance, except perhaps in cases of moderate resistance.

Removal of the lemma and palea reduced germination in each cultivar, as reported by Tapke (10) and Tervet (11). In our limited study, germination reductions of 5–50% were observed, depending on the cultivar. The effects on both germination and infection may be important

Table 7. Mean percentage of plants in oat cultivars inoculated with *Ustilago avenae* in tests from 1979 to 1984[y]

Cultivar	1979	1980	1981	1982	1983	1984	Mean
Benson	4.0	1.2	5.8	2.5	1.6	0.3	2.6 a
Lyon	1.8	0.5	1.6	0.5	0.1	0.3	0.8 a
Moore	15.6	10.0	19.2	13.4	26.7	8.5	15.6 b
Noble	1.4	0.2	0.3	0.2	0.0	0.0	0.4 a
Check[z]	52.0	46.7	48.3	54.3	58.7	49.1	51.5 c
Mean	14.9	11.7	15.1	14.2	17.2	11.6	

[y] Data are means of several observations for each cultivar each year: 5–20 observations per cultivar for 1979, 6–32 per cultivar for 1980, 6–13 per cultivar for 1981, 7–21 per cultivar for 1982, 3–15 per cultivar for 1983, and 3–21 per cultivar for 1984. Differences due to cultivars were significant but not those due to year of testing. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] The susceptible check was cultivar Marathon in 1979 and 1980, and Ogle in 1981 through 1984.

Table 8. Percentage of plants in differential oat cultivars infected with *Ustilago avenae* in different years

Cultivar	CI number	1979	1981	1986	1990
Anthony	2143	40	90	40	50
Black Diamond	1848	30	50	80	50
Black Mesdag	1877	40	60	50	60
Victory	560	50	90	50	50
Camas	2965	0	1	5	0
Markton	2053	0	0	5	2
Navarro	996	0	1	5	0
Fulgam	708	1	5	10	20
Monarch	1876	0	10	5	1
Nicol	2925	1	20	10	10
Clintland 64	7639	10	20	30	20
Gothland	1898	10	30	10	2
Victoria	2401	30	20	20	10

in studies on the genetics of resistance, as well as in cultivar selection.

In evaluating oat germ plasm for reaction to loose smut, we used an inoculum concentration that, in preliminary trials, was adequate to identify susceptible reactions. Although reasonable care was used in preparing the inoculum batches, it is possible that some variations in concentration occurred. However, experiments indicated that slight variations are of little importance in evaluating the smut reactions of oat cultivars and breeding lines.

Some cultivars were repeatedly evaluated for loose smut reactions as part of the routine testing procedure. Hazel, Moore, Ogle, and Steele were compared from 1985 to 1989; and Benson, Lyon, Moore, Noble, and susceptible checks Ogle and Marathon were compared from 1979 to 1984. Each year the incidence of infection varied by cultivar, but the cultivars did not change in rank relative to each other.

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