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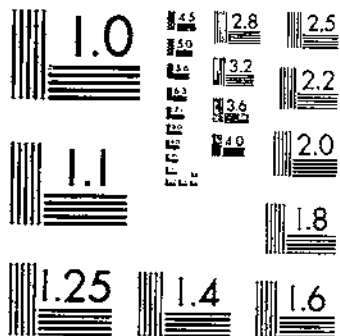
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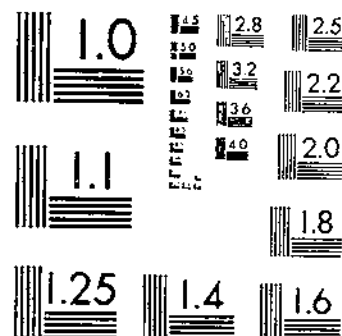
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SEED TREATMENT EXPERIMENTS WITH
OATS NATURALLY AND ARTIFICIALLY
INOCULATED WITH SMUTS¹

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INTRODUCTION

Liquid formaldehyde probably has been the most widely used fungicide for oat smut control since its introduction in 1897 by Bolley (1)³ and especially since the introduction of the dry-spray method by Haskell (6) in 1917. The dry method eliminated one of the objections to the use of formaldehyde for oat-seed treatment, namely, the thorough wetting of the seed. However, one other objection to this treatment remains. It frequently retards germination and reduces emergence, stand, and yield, especially when sowing is delayed after treatment, or when the seed is sown in soil too dry to induce immediate germination and growth (9). This retarded germination gives soil organisms an excellent opportunity to attack the seed. Consequently, ever since the development of dust fungicides for bunt control in wheat, efforts have been made to develop similar fungicides for the control of smuts in oats at a cost comparable to that of liquid formaldehyde and with less danger of seed injury. The frequent reports in recent years of satisfactory oat smut control with fungicidal dusts at a moderate cost and the increasing use of dust fungicides for this purpose seem to indicate that these efforts have met with success.

The original object of the experiments described in this bulletin was to test the effectiveness of a number of such disinfectants in the

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³ Italic numbers in parentheses refer to Literature Cited, p. 15.

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control of smuts in oats over a period of years. The lack at times of seed lots carrying a heavy natural infection made it necessary to use artificially inoculated seed. Several different methods of inoculating the seed were used. In addition to testing the merits of fungicides, therefore, the experiments afforded an opportunity to test the effectiveness of different methods of inoculating oats with smuts and to compare the results obtained with naturally and artificially inoculated seed in seed-treatment studies.

REVIEW OF LITERATURE

Copper carbonate frequently was tried as a fungicide in early experiments on oat smut control (2, 8, 12, 18, 19, 20, 22, 23, 24). With few exceptions, it was found to give unsatisfactory control of smut in hulled varieties and fair to good results in hull-less varieties. Such materials as mercuric chloride, copper acetate, copper sulphate, copper oxychloride, nickel carbonate, nickel sulphide, and sulphur, used alone or in various mixtures and dilutions, generally were reported impracticable (2, 4, 8, 12, 18, 22, 24). Iodine dust first reported as a promising fungicide (22) was found later to be generally ineffective (4, 7, 12, 25) in addition to possessing other qualities undesirable in a dust fungicide. Sayre and Thomas (22), in 1927, introduced formaldehyde dust, one of the most promising dust fungicides for oat smut control produced up to that time. In experiments subsequent to its introduction (10, 12, 21, 23, 25) certain commercial brands of this dust gave excellent control of oat smut as well as of barley covered smut (13, 15) and black loose smut (14, 15), but not barley stripe (16). Its use later as a soil disinfectant gave rise to the manufacture of certain commercial brands suitable for this purpose but not suitable for cereal-seed treatment. Formaldehyde dust has been found generally to be a safer treatment than liquid formaldehyde, although it may injure seed under certain conditions (13). Koehler (10) found that treating the seed with formaldehyde dust a week to 3 months before sowing caused a reduction in yield. In other experiments it caused no consistent increase in yields when applied to smut-free seed (17). Its rapid deterioration and loss of effectiveness unless kept in airtight sealed containers is one of its chief disadvantages. In 1928 Ceresan (ethyl mercuric chloride) was placed on the market and in a number of experiments (4, 10, 12, 18, 23, 25) proved very effective in oat smut control. Its chief disadvantage was its cost, which ranged from about 9 to 14 cents per bushel of seed treated. Preliminary experiments with ethyl mercuric phosphate were followed by its commercial production in 1933 as New Improved Ceresan. This dust has been found generally inexpensive and effective in control of oat smut and other cereal diseases (10).

MATERIAL AND METHODS

INOCULUM USED

Because loose smut and covered smut of oats are similar in their life histories (8) and in their reactions to seed disinfectants no attempt was made to carry on separate experiments with each species of smut. With one exception, both smuts were included in the same experiments, and in taking data their occurrence was recorded collectively. The inoculum used was a mixture of smuts obtained from different sections

of the country. Smuts from Lee, Kanota, Fulghum, and Victory oats grown on the Arlington Experiment Farm, Arlington, Va., near Washington, D. C., also were included. In one experiment with Fulghum oats, a virulent strain of covered smut from that variety was the only inoculum used. The smut was sifted through a 60-mesh sieve and kept in the refrigerator at 7° C. until used. The viability of the spores was determined by means of germination tests shortly before the inoculum was applied to the seed. The inoculum was obtained usually from the preceding crop and was, therefore, from 4 to 8 months old when used.

METHODS OF INOCULATION

Although most of the seed lots used were thought to be naturally inoculated, additional inoculum usually was applied in order to bring about heavier infection in the crop, and thus provide a more adequate test for the fungicides.

Some of the problems previously mentioned by the writer in connection with studies on the control of barley smuts (15) apply also to these similar studies on oat smuts. If seed secured from a heavily infected oat crop is sown it will not always produce another heavily infected crop. Weather conditions at blossoming time, not conducive to the opening of the flowers, are suggested by Gage (3) as an important controlling factor in the amount of infection, especially by loose smut, in the succeeding crop. He also intimates that if moisture and temperature conditions in storage are favorable, infection (of the seed) may take place from spores that reach the oats during the process of threshing. Therefore, in the experiments here described the seed usually was stored for some time before treatment under conditions favorable for the germination of the spores and for the development of the mycelium under the glumes. The inoculum was applied to the seed as dry spores at a 1- to 100-spore dosage (1 part by weight of spores to 100 parts of seed) or as a spore suspension in which the seed was immersed for 20 minutes under 35 inches of vacuum. The latter method of inoculation, first used in Germany (5) and generally referred to as the "evacuation method", was carried out as described in a previous article (15) except that the subsequent incubation temperature was held at 20° to 22° C. In experiment 1, with Lee oats, a spore suspension was made by adding 2 g of spores to a liter of culture solution containing 0.1 g each of dextrose, magnesium sulphate, sodium chloride, and calcium chloride, and 0.2 g each of ammonium sulphate, potassium sulphate, and potassium acid sulphate (5).

Another spore suspension was prepared by adding 2 g of spores to a liter of 2-percent dextrose solution. The seed inoculated with the latter spore suspension produced slightly more smut than that inoculated with the spores in the more complicated culture solution. Hence, in later experiments a 2-percent dextrose solution was used in preparing the spore suspension.

In experiment 2, with Kanota and Victory oats, half the seed was inoculated by immersing it in the spore suspension for 20 minutes without subjecting it to vacuum. In experiment 8, with Norton oats, separate lots of seed were subjected to different processes designed either to apply additional inoculum to the seed or to make the inoculum already present more effective in bringing about infection in the crop.

FUNGICIDES USED AND METHODS OF APPLICATION

The following materials were used as fungicides:⁴

- Ceresan, 2-percent ethyl mercuric chloride.
- New Improved Ceresan, 5-percent ethyl mercuric phosphate.
- Sanoseed, 5-percent ethanol mercuric chloride.
- Grainaide, organic mercury and methyl aldehyde.
- Smuttox, 4-percent formaldehyde in inert material.
- Ansul Dust, 6-percent formaldehyde in inert material.
- P. A. C. Dust, 6- to 8-percent formaldehyde in inert material.
- Corona Oat Dust, 5- to 7-percent formaldehyde in inert material.
- Corona 219, Corona Oat Dust plus 5 percent of hydroxy nitro mercury phenol.
- Corona 287, Corona Oat Dust plus 5-percent mercury ethoxy.
- Corona Compound "A", a rosin urea formaldehyde compound.
- Dubay 986—2-percent ethyl mercuric phosphate.
- S. K. 413-a, phenol-mercuric compound.
- Hongosan, a naphthalene compound.
- Formaldehyde solution, dip, and spray.
- Formacide, paraformaldehyde plus a catalytic agent in inert material.

The dust fungicides were applied to the seed at rates ranging from $\frac{1}{2}$ to 4 ounces per bushel in accordance with general practice or the recommendations of the manufacturers. The seed was then mixed with the dusts as described in a previous publication (15), after which it usually stood covered for at least 24 hours and was then stored in open containers or cloth sacks until sown. The lots of seed treated with the mercury and formaldehyde dusts were buried in larger lots of seed similarly treated with mercury and formaldehyde dusts, respectively. The formaldehyde spray treatment was applied by spraying the seed with a 1 to 1 dilution of commercial 37-percent formaldehyde solution at the rate of 1 quart of the mixture to 50 bushels (10 cc to 8,000 g of grain), after which the seed was buried in a larger bulk of seed similarly treated, covered for 5 hours, and then either sown at once or aired and stored. In applying the formaldehyde dip the seed was immersed in a 1 to 320 dilution of the commercial formaldehyde solution for 5 minutes, drained, covered 2 hours, dried, and either sown immediately or aired and stored.

In order to study the possible influence of temperature on the effectiveness of certain seed disinfectants in oat smut control (11) a quantity of Fulghum oats was inoculated by the evacuation method with a virulent strain of *Ustilago levis* (Kell. and Swing.) Magn. on March 22, 1935, incubated at 22° C. and 90 percent relative humidity for 24 hours, and then aired and stored for 3 weeks. Separate lots were then treated with New Improved Ceresan or Smuttox and portions of each lot stored at temperatures of 5°, 10°, 15°, 20°, and 25° for 4 days along with similar portions of untreated seed. The seed was then sown as uniformly as possible in 5-foot rows in well-prepared soil in outdoor beds. Separate portions of a fourth lot of seed were sprayed with portions of a 1 to 1 formaldehyde solution kept at the different temperatures mentioned, and the seed was then covered and stored at these respective temperatures for 5 hours before being sown.

SEED USED

Sixteen lots of seed embracing 11 varieties were used over a 5-year period, 1932-36. The different seed lots were obtained from eight different States and usually from smut-infected crops. They were thoroughly cleaned and graded before being used.

⁴ The sources of most of these compounds, the composition of which as here given was supplied by the manufacturers, have been given in a previous paper (15). Formacide is a product of the Hammond Paint & Chemical Co., Inc., Beacon, N. Y.

SOWING

In the field plots the seed was sown usually by hand in rod rows replicated a number of times. In the small outdoor beds the seed was sown in 5-foot rows at a uniform depth of 1½ inches and at the rate of 150 seeds per row. In the greenhouse, 100 seeds were sown 1½ inches deep in 3½-foot rows 1 foot apart. Data on emergence were obtained in the greenhouse and in the outdoor beds just before the appearance of the second leaf.

TAKING DATA

In the field plots and in the outdoor beds the smutted heads were counted in all the rows and also the total heads if any smut was present. Loose smut and covered smut usually were not recorded separately. In the greenhouse the plants were pulled and a record was made of healthy and smutted plants and heads. A plant bearing both healthy and smutted heads was counted as smutted.

EXPERIMENTAL RESULTS

The results obtained in the first experiment (1932) are presented in table 1. Five of the treatments eliminated smut, but two of these, Grainside and Corona 219, severely injured germination. Corona Oat Dust, 1 year old, was almost as effective as the freshly made material. Three of the treatments were more effective when sowing was delayed 8 days after treating. The fungicidal effectiveness of Corona Oat Dust was increased by the addition of a mercury nitrophenol compound (Corona 219) and decreased by the addition of mercury ethoxy (Corona 287).

TABLE 1.—Emergence of and loose and covered smuts in Sixty-Day oats grown from seed both naturally and artificially inoculated, dusted with different fungicides Mar. 18, 1932, stored in closed containers 18 hours, and sown in four series

[Series 1, sown outdoors Mar. 19, 450 seeds per treatment; series 2, sown outdoors Mar. 26, 100 seeds per treatment; series 3, sown in greenhouse Mar. 26, 100 seeds per treatment; series 4, sown in greenhouse Mar. 26, 100 seeds per treatment, after storage in closed container for 8 days]

Seed-treatment compound		Emergence in series--					Heads smutted in series--				
Name	Rate per bushel	1	2	3	4	Average†	1	2	3	4	Average†
	Oz.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Untreated.....		70	86	94	96	79	31.5	17.1	39.7	23.5	27.1
Ceresan.....	3	73	87	94	91	80	.0	.0	.0	.0	.0
Dubay 986.....	3	71	92	90	99	79	.0	.0	.0	.0	.0
Smutox.....	4	58	78	82	77	66	.0	.0	.0	.0	.0
Corona Oat Dust (1931).....	4	62	80	92	95	73	4.5	5.5	.0	.0	2.4
Untreated.....		69	85	90	95	71	25.5	8.4	52.8	41.2	28.1
Corona Oat Dust (1932).....	4	68	84	93	85	76	3.9	.0	.0	.0	2.1
Ansul Dust.....	4	63	85	92	87	72	1.0	.0	.0	1.4	.7
Corona 219.....	4	40	66	76	79	58	.0	.0	.0	.0	.0
Corona 287.....	4	63	93	93	73	75	31.2	3.6	7.0	9.9	19.0
Grainside.....	4	48	59	71	68	55	.0	.0	.0	.0	.0

† Weighted average.

The influence of environmental factors on smut development is demonstrated by the differences in the percentages of smut in the controls in the different series. The average temperature during the emergence of the seedlings in the two outdoor series, series 1 and 2,

was about 15° C., but the second seeding received a heavy rain. The seedlings in the greenhouse, series 3 and 4, were grown to emergence at an average temperature of 20° and in a drier soil. The effects of temperature and soil moisture are apparent.

In the second experiment, conducted in 1932-33, Fulghum oats, artificially inoculated with dry smut spores, were treated with Ceresan, Smuttox, Corona Oat Dust, Ansul Dust, Grainaide, Sanoseed, Dubay 1100, S. K. 413-a, and formaldehyde spray and dip. Data of value on smut control were not obtained because of severe winter-killing. Germination was adversely affected by Grainaide, Dubay 1100, and the formaldehyde treatments. The treatments did not seem to affect in any way the degree of winter-killing, but the plants grown from seed not artificially inoculated, or inoculated and treated, survived better than those grown from inoculated untreated seed. Zade (26) reported that infected plants are more susceptible to winter-killing than are noninfected plants.

The data on smut development and control in Lee oats, inoculated, treated, and sown in September 1933 are presented in table 2.

TABLE 2.—Loose and covered smuts in Lee oats grown from seed both naturally and artificially inoculated, treated with different fungicides, and sown Sept. 27, 1933, with a hand nursery drill in 65-foot rows replicated twice for each treatment and for each of three methods of artificial inoculation

[Series 1, seed inoculated with spores suspended in nutrient solution in vacuum; series 2, same as series 1, but using 2-percent dextrose solution; series 3, seed dusted with dry spores]

Seed-treatment compound		Heads in series 1		Heads in series 2		Heads in series 3	
Name	Rate per bushel	Total	Smutted	Total	Smutted	Total	Smutted
	Ounces	Number	Percent	Number	Percent	Number	Percent
Untreated		6,260	40.7	6,610	41.6	6,020	28.9
Ceresan	3	5,580	.4	6,280	2.4	6,070	.0
New Improved Ceresan	1 ¹ / ₂	5,350	.4	6,200	1.6	6,500	.0
Sanoseed	3	5,040	15.5	6,000	34.3	6,180	2.2
Grainaide	3	5,940	3.2	6,200	4.0	6,130	.0
Smuttox	3	6,420	3.6	5,790	15.9	6,050	.0
Untreated		5,620	36.0	5,930	42.2	6,900	26.6
Ansul Dust	3	5,020	33.6	6,140	37.0	6,840	32.7
P. A. C. Dust	3	5,500	2.4	4,820	3.3	5,000	.2
S. K. 413-a	3	6,240	10.3	6,600	20.7	5,850	1.0
Hongosol	3	5,600	40.9	6,100	41.6	5,800	23.9
Formaldehyde (1:320)	(¹)	6,450	2.2	6,080	6.2	6,140	2.1
Uninoculated, untreated		6,540	6.5	6,400	6.6	7,220	6.5

¹ Seed immersed in a 1 to 320 solution 5 minutes, drained, and covered 2 hours.

Inoculating the seed by the evacuation method and using a culture solution for the spore suspension, as described by Haarring (5), resulted in 38.4 percent of smutted heads in the controls and an average of 2 percent from seed treated with the six better fungicides. When a 2-percent dextrose solution was used for the spore suspension the corresponding percentages were slightly higher, namely, 41.9 and 5.6, respectively. Inoculation of the seed with dry spores resulted in only 26.8 percent of smutted heads in the controls and an average of 0.4 percent from seed treated with the six best fungicides. Apparently there was about 6.5 percent of smut due to natural inoculation. This smut, along with that due to the application of dry spores to the seed, evidently was more easily controlled than was the smut brought about by the evacuation method of inoculation, as only three of the dusts reduced the percentage of smut to less than 4 percent in series 2.

The naturally inoculated seed of Fulghum and Colorado No. 37 oats treated March 26, 1934, and sown in the greenhouse and out of doors March 27 and April 2, respectively, produced the results presented in table 3.

TABLE 3.—Germination and smut control in Fulghum and Colorado No. 37 oats grown from naturally inoculated seed, treated with various fungicides, and sown in two series, 1934

[Series 1, in greenhouse benches, Mar. 27; series 2, in outdoor plots, Apr. 2]

Variety and seed-treatment compound		Series 1		Series 2		
Name	Rate per bushel	Germination	Smutted heads		Smutted heads	
			Number	Percent	Number	Percent
Fulghum:	<i>Ounces</i>	<i>Percent</i>				
Untreated		53	18	12.8	180	20.0
Ceresan	3	89	0	.0	0	.0
New Improved Ceresan	3 ¹	86	0	.0	0	.0
Sanoseed	3	92	1	.5	14	1.6
Grainaide	3	92	0	.0	0	.0
Smuttox	3	93	1	.8	2	.2
Untreated		80	16	13.0	255	28.0
Ansul Dust	3	89	12	8.8	317	22.6
P. A. C. Dust	3	90	0	.0	0	.0
S. K. 413-a	3	81	2	1.2	8	.8
Hongosan	3	85	16	11.2	120	13.3
Formaldehyde (1:320)	(1)	96	0	.0	0	.0
Smutted and untreated		64	30	60.0	240	40.5
Colorado No. 37:						
Untreated		58	9	10.7	64	9.4
Ceresan	3	70	0	.0	0	.0
New Improved Ceresan	3 ¹	68	0	.0	0	.0
Sanoseed	3	75	2	2.1	0	.0
Grainaide	3	60	1	1.0	1	.2
Smuttox	3	67	0	.0	2	.3
Untreated		44	17	22.7	65	12.0
Ansul Dust	3	62	7	7.5	73	13.5
P. A. C. Dust	3	85	3	3.2	1	.2
S. K. 413-a	3	79	0	.0	0	.0
Hongosan	3	63	13	15.7	41	7.5
Formaldehyde (1:320)	(1)	87	0	.0	1	.2
Smutted and untreated (dry spores)			34	37.0	233	20.1

¹ Seed immersed in a 1 to 320 solution 5 minutes, drained, and covered 2 hours.

Most of the dusts improved germination, especially in Colorado No. 37. Only two dusts eliminated smut in both varieties in both indoor and outdoor trials. Four other dusts and formaldehyde liquid reduced the infection to a weighted average of less than 1 percent. Ansul Dust and Hongosan were relatively ineffective. Environment seemed to influence smut development differently in the two varieties. In Fulghum infection was higher in the outdoor series, series 2, and in Colorado No. 37 it was greater in the indoor series, series 1. In Fulghum, applying smut spores to the seed increased smut infection relatively more than it did in Colorado No. 37 in both sowings. The plant population in the greenhouse was too small, however, to give much significance to the results obtained in that series.

In table 4 are presented the germination and infection data obtained in 1935 from Lee oats grown from seed inoculated by the evacuation method and by the dry-spore method, and, after treatment, sown in three series—(1) at Statesville, N. C., and (2) and (3) on the Arlington Experiment Farm in small outdoor beds of rich soil and in field plots, respectively.

TABLE 4.—Effect of different inoculation methods and seed treatments on germination and smut occurrence in Lee oats sown in three series, 1934-35

[Series 1, sown at Statesville, N. C., Oct. 1; series 2, at the Arlington Experiment Farm in rich soil, Oct. 12; series 3, at the Arlington Experiment Farm in poor soil, Oct. 1]

Method of inoculation	Seed-treatment compound		Germination after storage for—		Smutted heads in series—			Total smutted heads	
	Name	Rate per bushel	8 days	5 months	1	2	3		
			Ounces	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Number
Spore suspension in vacuum.	Uninoculated, untreated	94	99	7.8	16.2	30.8	533	13.0	
	Inoculated, untreated	94	94	42.0	67.1	47.9	2,110	55.8	
	New Improved Ceresan.	3 1/2	80	64	.2	.2	6	.2	
	Smutttox	3	91	85	.4	.8	3	.1	
	P. A. C. Dust	3	92	84	3.3	3.5	5.0	148	4.2
	Ansul Dust	3	92	88	4.4	6.4	4.9	202	5.5
	Formaldehyde	(1)	88	93	2.2	.5	.2	7	.3
	do.	(2)	94	82	2.0	5.0	2.3	132	3.3
	Uninoculated, untreated	94	98	7.8	16.6	14.7	490	14.0	
	Inoculated, untreated	90	95	23.3	37.9	30.0	1,122	33.5	
Dry spores	New Improved Ceresan.	1 1/2	82	62	.0	.0	1	.1	
	Smutttox	3	89	84	.2	1.0	12	.5	
	P. A. C. Dust	3	90	92	.2	.5	1.4	.8	
	Ansul Dust	3	94	93	1.2	2.7	4.0	98	2.9
	Formaldehyde	(1)	91	81	.0	.0	.1	.2	
	do.	(2)	95	85	.4	3.0	2.0	66	2.0

¹ Sprayed with a 1 to 1 solution, covered 5 hours, and then aired.² Dipped in a 1 to 320 solution 5 minutes, covered 2 hours, washed in water, and dried.

As in the previous year's experiments, the evacuation method of inoculation caused a higher average percentage of infection (55.8) than did the dry-spore method (33.5). The former method again seemed to induce a more deeply seated seed infection that was less successfully eliminated by the disinfectants.

Washing the seed in water after it had lain covered for 2 hours following a dip in a 1 to 320 formaldehyde solution greatly reduced the usual effectiveness of that treatment. Germination was adversely affected by the formaldehyde spray and by New Improved Ceresan. The formaldehyde dusts also caused some reduction in germination after the seed had been stored for 5 months. Lack of sufficient aeration during storage and a relatively high moisture content of the seed probably accounts for some of this injury.

Data from the Kanota and Victory oats inoculated March 11, 1935, by means of a spore suspension with and without vacuum, are presented in table 5.

The evacuation method of inoculation with a spore suspension resulted in an average of 26.1 and 46.6 percent of smut in Kanota and Victory, respectively, while the use of the same spore suspension without the vacuum produced only 9.8 and 10.6 percent of smut, respectively.

The formaldehyde dusts, which had been kept in supposedly tightly closed containers for 5 months, were, on the whole, unsatisfactory in the control of smuts in these varieties. New Improved Ceresan was the only treatment used that controlled smut without injury to the seed. Corona Compound "A" and the formaldehyde treatments eliminated smut but greatly reduced the stand of grain.

TABLE 5.—Effect of different inoculation methods and seed treatments on emergence and smut¹ occurrence in 2 varieties of spring oats sown in 4 series, 1935

[Series 1, in outdoor beds, Mar. 21; series 2, in field plots, Mar. 21; series 3, in field plots, Mar. 30; series 4, in the greenhouse, Apr. 9]

Variety and method of inoculation	Seed-treatment compound		Germination ¹	Smutted heads in series—				Total smutted heads		
	Name	Rate per bushel		1	2	3	4	Number	Percent	
Kauota:		Ounces	Percent	Percent	Percent	Percent	Percent	Number	Percent	
	Spore suspension in vacuum.	Uninoculated, untreated	90	0.0	0.0	0.0	0.0	0.0	1,810	0.2
		Inoculated, untreated	72	38.0	22.3	3.4	60.0	1,834	26.1	
		New Improved Ceresan	3 ^{1/2}	87	4.4	0.0	0.0	0.0	1,697	1.1
		Smutttox	3	88	13.3	3.4	0.0	6.1	1,710	0.1
	Spore suspension without vacuum.	P. A. C. Dust	3	37	0.0	0.0	0.0	7.7	1,001	7.2
		Corona Compound "A"	(3)	30	0.0	0.0	0.0	0.0	741	0.0
		Formaldehyde spray	(3)	30	0.0	0.0	0.0	0.0	757	0.0
		Formaldehyde dip	(3)	30	0.0	0.0	0.0	0.0	757	0.0
	Spore suspension in vacuum.	Uninoculated, untreated	90	0.0	2.2	0.0	0.0	0.0	1,715	1.1
		Inoculated, untreated	84	18.1	5.8	0.0	15.2	1,596	9.8	
		New Improved Ceresan	1 ^{1/2}	91	0.0	0.0	0.0	0.0	1,483	0.0
		Smutttox	3	73	10.4	1.0	0.0	0.0	1,473	3.0
	Spore suspension without vacuum.	P. A. C. Dust	3	76	9.4	1.0	0.0	0.0	1,493	3.6
		Corona Compound "A"	3	43	0.0	0.0	0.0	0.0	1,042	0.0
		Formaldehyde spray	3	17	0.0	0.0	0.0	0.0	573	0.0
Formaldehyde dip		3	41	0.0	0.0	0.0	0.0	806	0.0	
Victory:		Ounces	Percent	Percent	Percent	Percent	Percent	Number	Percent	
	Spore suspension in vacuum.	Uninoculated, untreated	90	0.0	0.0	0.0	0.0	0.0	1,127	0.0
		Inoculated, untreated	59	58.6	45.4	12.9	87.7	1,044	46.6	
		New Improved Ceresan	3 ^{1/2}	82	0.0	0.0	0.0	0.0	1,108	0.0
		Smutttox	3	73	4.4	1.4	0.0	5.9	1,065	2.5
	Spore suspension without vacuum.	P. A. C. Dust	3	72	5.0	0.9	0.0	7.3	1,073	3.0
		Corona Compound "A"	3	39	0.5	0.0	0.0	0.0	670	0.0
		Formaldehyde spray	3	28	0.0	0.0	0.0	0.0	571	0.0
		Formaldehyde dip	3	32	0.0	0.0	0.0	0.0	675	0.0
	Spore suspension without vacuum.	Uninoculated, untreated	70	0.0	0.0	0.0	0.0	0.0	1,090	0.0
		Inoculated, untreated	63	15.5	11.4	1.6	13.4	1,039	10.6	
		New Improved Ceresan	1 ^{1/2}	71	0.0	0.0	0.0	0.0	1,104	0.0
		Smutttox	3	75	4.9	5.3	0.0	0.0	976	3.4
	Spore suspension without vacuum.	P. A. C. Dust	3	73	10.9	9.1	0.0	0.0	1,024	6.6
		Corona Compound "A"	3	37	0.0	0.0	0.0	0.0	675	0.0
		Formaldehyde spray	3	10	0.0	0.0	0.0	0.0	291	0.0
Formaldehyde dip		3	50	0.0	0.0	0.0	0.0	663	0.0	

¹ From 25 to 40 percent of the smut was covered smut.² Seed for germination test was sown 20 days after treatment.³ Soaked in a 1 to 10 solution 5 minutes, drained, covered 2 hours, and dried.⁴ Seed sprayed with a 1 to 1 solution, covered 5 hours, and then dried.⁵ Dipped in a 1 to 320 solution, drained, covered 2 hours, and dried.

In table 6 are presented the data from Fulghum oats inoculated with a virulent strain of covered smut treated and stored as previously described and sown in outdoor beds.

TABLE 6.—Covered smut in Fulghum oats grown from seed artificially inoculated Mar. 23 with a spore suspension of *Ustilago levis* by the evacuation method, treated Apr. 11¹ at different temperatures, and sown in outdoor beds Apr. 15, 1935

Seed-treatment compound		Heads infected following treatment at—										Total heads smutted	
Name	Rate per bushel	5° C.		10° C.		15° C.		20° C.		25° C.		No.	Pct.
		No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.		
Control		598	56.3	505	60.1	535	62.7	569	65.8	556	59.7	2,074	60.9
New Improved Ceresan	3 ^{1/2}	38	4.4	33	3.9	37	4.4	33	3.8	37	5.9	200	4.5
Smutttox	4	2	.3	3	.4	5	.6	3	.4	1	.1	14	.3
Formaldehyde ²	4	4	.5	1	.1	3	.4	2	.3	0	.0	10	.2

¹ Formaldehyde treatment applied Apr. 15.² Sprayed with a 1 to 1 solution and covered 5 hours before sowing.

The temperature at which the grain was stored after the treatments were applied had little apparent effect on the relative efficacy of the fungicides. Smuttox, which had failed to give adequate control of smuts in Kanota and Victory in the previous experiment (table 5), allowed an average of only 0.3 percent of covered smut to appear in Fulghum. On the other hand, a fresh lot of New Improved Ceresan received immediately before being used permitted from 3.8 to 5.9 percent to appear, or an average of 4.5 percent, although another lot of New Improved Ceresan a few months old had furnished excellent control of smuts in Kanota and Victory in the previous experiment.

Formaldehyde spray, applied 5 hours before sowing the seed, was the most effective treatment used. Representing the stand from untreated seed as 100, the relative stands from seed treated with New Improved Ceresan, Smuttox, and formaldehyde were 101, 92, and 94, respectively.

The experiment with seed of naturally inoculated Norton oats (1935-36) yielded no highly significant data because of the low percentage of smutted heads obtained in the controls from untreated seed. The different methods of artificial inoculation used in this case had little effect on the subsequent percentages of infection, which were uniformly low probably because the conditions prevailing shortly after sowing were unfavorable for oat-smut development. It also is possible that the smut used was of low viability or that the Norton variety of oats was somewhat resistant to it. Furthermore, winter-killing was severe and most of the plants that succumbed may have been those that were infected.

All the dusts, with the exception of the 1-year-old Ansul Dust, controlled smut satisfactorily (table 7), but they also caused some reduction in germination, especially the fresh formaldehyde dusts.

TABLE 7.—Germination and smut control in Norton oats grown from naturally inoculated seed, separate lots of which had been subjected to different methods of artificial inoculation 2 weeks before treatment with different fungicides and sown in field plots, 1935-36

Seed-treatment compound			Germination ¹	Rod rows	Heads		
Name	Rate per bushel	Age			Total	Smutted	
	Ounces	Months	Percent	Number	Number	Percent	
Untreated			90	40	11,514	1,915	16.6
New Improved Ceresan	1/2	12	87	16	4,070	0	.0
Do.	1	1	85	40	12,510	11	.1
Smuttox	3	12	82	18	3,530	4	.1
Do.	3	14	77	40	10,615	0	.1
Ansul Dust	3	12	85	16	3,430	308	9.0
Do.	3	1/2	77	40	11,360	2	.1
P. A. C. Dust	3	12	70	16	3,240	4	.1
Do.	3	14	57	16	2,560	0	.0
Formaldehyde	(?)		78	40	11,240	2	.1

¹ Data obtained from plants grown in outdoor beds from seed sown 2 weeks after treatment.

² Trace.

³ Dipped in 1 to 320 formaldehyde 5 minutes, drained, and covered 2 hours.

The fact that two of the lots of formaldehyde dust a year old controlled smut about as well as the fresh material indicates that these dusts will retain their effectiveness if properly stored in sealed containers.

TABLE 8.—*Smut occurrence and control in spring oats as affected by method of inoculation, seed treatment, interval between treating and sowing, and soil temperature during period of emergence, 1935-36*

Seed-treatment compound ¹	Average soil temperature	Date seed was—		Percentage of smut infection ² from seed inoculated by—												
				Spore suspension in vacuum						Dry spores						
		Treated	Sown	Kanota	Iogold	Victory	Swedish Select	Ithacan	Iomine	Average ³	Kanota	Iogold	Victory	Swedish Select	Average ³	
	°C.															
Untreated	22	Dec. 6	Dec. 7 (greenhouse)	69	59	89	57	39	79	65	36	7	42	28	28	
New Improved Ceresan				51	4	62	33	27	15	32	1	1	1	0	0	.8
Smuttox				51	43	64	53	37	48	49	.0	0	.0	0	0	.0
Formacide				31	32	60	30	16	37	34	.0	0	.0	0	0	.0
Untreated	21	do	Feb. 24 (greenhouse)	61	52	94	58	34	56	59	39	11	64	21	34	
New Improved Ceresan				2	3	24	7	2	1	7	.0	0	.0	0	0	.0
Smuttox				8	21	59	27	8	14	23	.0	0	.0	0	0	.0
Formacide				18	16	52	26	6	9	21	.0	0	2	0	0	.5
Untreated	12	do	Mar. 30 (outdoors)	34	24	62	26	8	37	32	9	1	12	9	8	
New Improved Ceresan				2	0	10	1	0	2	6	.0	0	.0	0	0	.0
Smuttox				4	3	22	5	1	2	5	.0	0	.0	0	0	.0
Formacide				2	4	15	8	0	1	5	.0	0	.0	0	0	.0
Untreated	20	Mar. 4	Mar. 6 (greenhouse)	50	49	88	58	41	67	57	32	13	51	30	30	
New Improved Ceresan				7	2	24	12	14	2	10	.7	0	.0	0	1	.2
Smuttox				26	28	49	45	23	31	33	.0	0	3	.0	1	.0
Formacide				17	16	42	25	15	23	22	.0	0	.0	0	0	.0
Untreated	12	do	Mar. 30 (outdoors)	41	23	64	30	6	26	32	13	2	15	3	3	
New Improved Ceresan				.7	0	7	1	1	0	6	.0	0	.7	0	0	.2
Smuttox				8	6	12	4	2	5	6	.0	0	.7	0	0	.0
Formacide				2	7	13	6	3	2	6	1	0	.0	0	0	(5)

¹ New Improved Ceresan was applied at ½ ounce per bushel; Smuttox and Formacide each at 3 ounces per bushel.

² In indoor plantings infection was based on plant counts; in outdoor plantings, on head counts.

³ Weighted averages.

⁴ Trace.

During the year 1935-36 seed lots of six varieties of oats were inoculated by the evacuation method and separate lots of seed of four of the same varieties were inoculated with dry spores. A month later, portions of all of these lots were treated with New Improved Ceresan, Smuttox, and Formacide and the following day sowings were made in the greenhouse bench. The development of the plants was hastened by extending the normal period of daylight with electric lights so that data on smut occurrence were taken in February. The surprisingly high percentages of smutted plants from treated seed that previously had been inoculated by the evacuation method (table 8) was attributed to the short time (23 hours) between treating and sowing. Therefore, on February 24 another sowing from these same portions of treated seed was made in the greenhouse--80 days after treatment. On March 4 additional portions of the different lots of inoculated seed were treated with the same materials used before, extreme care being taken as to rate and method of application. Sowings were made in the greenhouse on March 6 and in outdoor beds on March 30, the outdoor sowing also including seed that had been treated December 6.

The data in table 8 show that environmental conditions undoubtedly were more conducive to smut development in the greenhouse than they were outdoors. They also show that the longer the period between treatment and sowing, the better was the smut control both in the lots treated December 6 and in those treated March 4. This applies particularly to the seed that had been inoculated by the evacuation method.

TABLE 9.—Effect of seed treatments on emergence and stand in six varieties of spring oats, 1935-36

Variety	Average of emergence and stand from seed—							
	Untreated		Treated with—					
			New Improved Ceresan		Smuttox		Formacide	
	Emergence	Stand	Emergence	Stand	Emergence	Stand	Emergence	Stand
Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	
Kanota.....	95	91	98	93	97	97	96	95
Jagold.....	92	81	96	90	92	90	90	86
Victory.....	89	74	93	91	80	81	80	73
Swedish Select.....	91	83	95	85	90	89	87	85
Ithacan.....	96	90	97	93	96	92	91	91
Iomlino.....	92	89	93	92	92	91	91	88
Average.....	93	84	96	95	94	89	91	85

Data on emergence and stand are presented in table 9. Smuttox and Formacide at times injured germination, especially in the Victory oats, the moisture content of which had been increased by immersion in the spore suspension during the process of inoculation. The relatively heavy glumes of this variety probably were conducive to the retention of much of this moisture by the seed. The seedlings of Victory oats also were attacked by a seedling blight against which excellent protection seemed to be furnished by New Improved Ceresan, less by Smuttox, and apparently none by Formacide, as shown by the data on emergence and stand in table 9.

DISCUSSION

To facilitate a comparison between some of the results obtained from the different materials and methods employed in the foregoing experiments, there are given in table 10 the names of the varieties of oats used, their sources, the methods and dates of inoculation, and the dates on which the different lots of seed were treated and sown, together with the average percentages of infection obtained from seed subjected to different methods of inoculation and either treated or not treated.

Probably the outstanding feature of the foregoing experiments is the relatively high percentage of smut usually obtained by means of the evacuation method of inoculation as compared with the dry-spore method, and also the occasional failure of the better disinfectants to satisfactorily eliminate this infection.

The writer can offer no satisfactory explanation for these poor results with disinfectants, which in other experiments had furnished fairly good smut control. It is possible that the evacuation method of inoculation may have caused the glumes upon drying to adhere more closely to the caryopsis, thus tending to shut out the fumes of the disinfectants used. However, an examination of a large number of kernels of the four varieties inoculated either by the evacuation or dry-spore method in the last experiment failed to convince the writer that this was the case. It did reveal the fact, however, that the evacuation method of inoculation had literally darkened the caryopsis and inner side of the glumes with spores and that the latter were particularly abundant about the embryo end. On the other hand, no spores were found beneath the glumes of the seeds that had been inoculated with dry spores, although in the latter case spores were much more abundant on the outside of the glumes.

About 25 kernels from each of the six lots of Norton oats used in experiment 8 also were examined under a dissecting microscope. The uninoculated kernels showed a sprinkling of spores on the outside of the hulls, but none was found on the caryopsis except where the brush end was exposed. The seeds inoculated with dry spores carried a heavy spore load on the outside of the glumes, but practically no spores were found beneath the glumes. The seeds that had been immersed in a spore suspension but not evacuated showed a generous sprinkling of spores on the caryopsis, especially near the brush end. In a few cases some spores had almost reached the embryo end. In the evacuated seeds, however, the spores were most abundant about the embryo end of the caryopsis and many had been forced into the groove and into the irregularities of the pericarp about the embryo. In the lots immersed in water, with and without vacuum, a few spores were found on the outer sides of the glumes, but only rarely were spores found on the pericarp or on the inner sides of the glumes.

The fact that the application of inoculum to the seed of Norton oats did not materially increase the percentage of infected heads in this variety seems to indicate that the smut that developed in the crop must have been caused by natural inoculation of the seed at blossoming time, as described by Gage (3). Lack of viability in the smut used for inoculum, or varietal resistance to it, may account for its failure to produce more infection.

It seems that the evacuation method of inoculation, if properly used, should be of value not only for seed treatment work but for studies on varietal resistance and physiologic forms. The inoculated seed should be allowed to dry thoroughly before applying dust fungicides, or seed injury may follow.

TABLE 10.—Names and sources of oat varieties used in experiments on smut control, together with information concerning inoculation, treatment, and sowing of the seed, and certain data on infection, 1932-36

Experiment no.	Table no.	Seed used—			How inoculated	Incubated	Date seed was—			Average of infection from seed—			
		Variety	Source	Year grown			Inoculated	Treated	Sown	Not inoculated		Inoculated	
										Un-treated	Treated ¹	Un-treated	Treated ¹
1	1	Sixty-Day	Urbana, Ill.	1931	Dry spores	0	Mar. 4	Mar. 18	Mar. 19-26			26.6	0.7
2		Fulghum	Arlington Farm, Va.	1932	do.	0	Sept. 15	Sept. 25	Oct. 1				(²)
3	2	Lee	do.	1933	Evacuation ³	40	Sept. 20	do.	Sept. 27		6.5	38.4	3.2
					do.	40	do.	do.	do.		6.5	41.9	7.7
					Dry spores	40	do.	do.	do.		6.5	26.8	.6
		Fulghum	Hays, Kans.	do.	None	0	do.	Mar. 26	Mar. 27		12.9	0.4	60.0
		do.	do.	do.	do.	0	do.	do.	Apr. 2		23.0	.3	40.5
4	3	do.	do.	do.	do.	0	do.	do.	Mar. 27		16.7	.8	37.0
		Colorado No. 37	Akron, Colo.	do.	do.	0	do.	do.	Apr. 2		10.7	.3	29.1
		do.	do.	do.	do.	0	do.	do.	do.				
5	4	Lee	Arlington Farm, Va.	1934	Evacuation	72	Sept. 7	Sept. 23	Oct. 1 and 12		11.3		55.8
					Dry spores	72	do.	do.	do.		11.3		33.5
		Kanota	Hays, Kans.	do.	Evacuation	48	Mar. 1	Mar. 20	Mar. 21				26.1
		do.	do.	do.	do.	48	do.	do.	do.				9.8
6	5	Victory	Dickinson, N. Dak.	do.	Spore suspension	48	do.	do.	do.				46.6
		do.	do.	do.	Evacuation	48	do.	do.	do.				10.6
		do.	do.	do.	Spore suspension	48	do.	do.	do.				60.9
7	6	Fulghum	Arlington Farm, Va.	do.	Evacuation ³	24	Mar. 22	Apr. 11	Apr. 15				
					None	24	Sept. 9	Sept. 20	Oct. 2		15.1	.0	
					Dry spores	24	do.	do.	do.		15.1		19.0
					Spore suspension	24	do.	do.	do.		15.1		14.7
8	7	Norton	Statesville, N. C.	1935	Evacuation	24	do.	do.	do.		15.1		14.5
					Water only	24	do.	do.	do.		15.1		22.3
					Water under vacuum	24	do.	do.	do.		15.1		19.5
		Kanota	Hays, Kans.	do.	Evacuation	48	Nov. 7				8.0		51
		Iogold	Ames, Iowa	do.	do.	48	do.				.0		41
		Victory	Dickinson, N. Dak.	do.	do.	48	do.	Dec. 6 and Mar. 4	Dec. 7, Feb. 24, Mar. 6, and Mar. 30.		.5		79
		Swedish Select	do.	do.	do.	48	do.	do.	do.		.0		46
		Ithaca	Ithaca, N. Y.	do.	do.	48	do.	do.	do.		.0		26
		Iomine	Ames, Iowa	do.	do.	48	do.	do.	do.		.0		53
		Kanota	Hays, Kans.	do.	Dry spores	72	do.	do.	do.		8.0		26
		Iogold	Ames, Iowa	do.	do.	72	do.	do.	do.		.0		7
9	8 and 9	Victory	Dickinson, N. Dak.	do.	do.	72	do.	do.	do.		.5		37
		Swedish Select	do.	do.	do.	72	do.	do.	do.		.0		17

¹ Results from the more ineffective treatments are omitted.
² No infection data obtained because of severe winter-killing.
³ The spore suspension in this case was a complex culture solution.

⁴ Inoculated by the dry-spore method without special incubation.
⁵ In this experiment covered smut only was used.

With the exception of the last experiment, control of oat smut was generally satisfactory with the better dust fungicides. The occasional failure of the formaldehyde dusts to effect satisfactory control must be attributed to the loss of volatile matter from these materials upon standing. Liquid formaldehyde and formaldehyde spray were effective but frequently injurious to the seed. New Improved Ceresan with one exception was, on the whole, the most satisfactory fungicide used in these experiments, both from the standpoint of smut control and effect on germination and stand. Formacide, although included in only one experiment, gives promise of being a good disinfectant for oats. If bought in 100-pound lots its cost comes to about 3.4 cents per bushel of seed, which is about the same as for formaldehyde dusts, although somewhat higher than for New Improved Ceresan. The other materials used were, on the whole, unsatisfactory or not of sufficient promise to merit discussion.

SUMMARY

In experiments with 11 varieties of oats over a 5-year period higher percentages of smut usually resulted from seed inoculated by a spore suspension under vacuum than from seed inoculated similarly without vacuum or with dry spores, or from naturally inoculated seed.

The smut infection caused by the evacuation method of inoculation was, at times, less amenable to control by disinfectants than was that caused in other ways.

Oat smuts developed most abundantly in soil with a low to medium moisture content and at a temperature of about 20° C.

The treatments that controlled oat smuts most satisfactorily were those with New Improved Ceresan, formaldehyde dip or spray, certain formaldehyde dusts, and Formacide, a paraformaldehyde dust containing a catalytic agent, which, in the presence of moisture, causes paraformaldehyde to revert to gaseous formaldehyde. The dusts were more effective if applied 2 or more days before sowing. All of them injured germination at times when the treated oats were stored too long without proper aeration, or when the seed had a relatively high moisture content. Prolonged storage of oats treated with the above dust fungicides is not recommended unless the grain has a moisture content of about 14 percent or less, is thoroughly aerated a few days after treatment, and the storage place is cool and dry.

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