THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Edmund Bryan Lambert for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

E C Stakman
Chairman

J. J. Willaman

H. K. Hayes

Date May 16, 1922

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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Edmund Bryan Lambert final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

May 6 1922

E C Stakman
Chairman

J. J. Willaman

H. K. Hayes

THE EFFECT OF CHEMICAL DUSTS ON CEREAL SMUTS

A thesis presented to the Faculty of the
Graduate School of the University of
Minnesota in partial fulfillment
of the requirements for the
Degree of Master of
Science

By

Edmund B. Lambert

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THE EFFECT OF CHEMICAL DUSTS ON CEREAL SMUTS

INTRODUCTION

The necessity for an effective method of controlling smut in grain has been felt for over a century. DeBary (7) notes that, "In 1783 Tessier stated that he had demonstrated the infectious nature of stinking smut of wheat by experiments", and that Elsner stated in 1821 that, "Volumes might be filled with all that had been written about smuts. Experimentation and observations, however, were badly lacking. This might account for the fact that among all of the writers no two opinions harmonized."

Prevost (13) in 1807 treated wheat with a solution of copper sulphate in order to prevent stinking smut. He recommended the use of six percent of copper sulphate in water and claimed that this was an infallible preventive, superior to anything else. This method of treating grain for the prevention of smut, however, was not used generally until modified and recommended by Kühn (10) in the middle of the nineteenth century.

Jensen (9), in 1888, published the first of a series of papers giving the results of experiments by which he showed the smuts could be prevented by treating seed in hot water.

In 1890 Arthur successfully used formadehyde as a germicide in treating grain. Bolley (1) popularized this method of treating seed and formaldehyde became, for the next quarter of a century, the most popular fungicide used for the prevention of smut in this country.

Evidence of the harmful effects of treating grain with copper sulphate or formaldehyde solutions has been accumulating since the beginning of seed treatment practices. Modern research therefore has sought to determine the nature of this seed injury and to lessen it by modifications in the methods of treatment.

Coons (3) notes that seed which has been treated with formaldehyde is injured less if it is planted in moist soil than if it is planted in dry soil. Hurd (8) showed that the injury is greater when seed which has been treated with formaldehyde is dried quickly than when it is dried slowly. Braun found that presoaking the seed in water before immersing it in either formaldehyde or copper sulphate reduced the amount of seed injury. Zundel (14), after citing forty-five workers who had observed seed injury resulting from the use of formaldehyde or copper sulphate, stated that, "The most practical method for preventing seed injury is to dip the wheat in lime water after treating with copper sulphate or formaldehyde."

These methods, although effective in preventing seed injury, are cumbersome, and would be impractical on a large scale.

Darnell-Smith (4), Biologist of the Department of Agriculture of New South Wales, Australia, in 1915 and 1916, prevented covered smut in winter wheat by dusting the seed with copper carbonate dust at the rate of four ounces per bushel. In 1919 Darnell-Smith and Ross (6) published the results of more extensive experiments in which they compared the dry copper carbonate method with the bluestone method. The results of these tests were even more favorable than those of the previous ones. The plots sown with seed treated by the dry method yielded much better than did any of the others. In 1920 Mackie and Briggs (11) tested the effects of copper dusts on winter wheat in California and they also obtained absolute control of smut with the use of copper carbonate dust.

The results of these experiments were so promising that it seemed desirable to compare the effectiveness of dust treatments with the standard formaldehyde methods of treating grain in the hard red spring wheat region.

OBJECTS OF THE INVESTIGATION

The necessity for treating seed of the common cereals to prevent smuts is clearly indicated by the following table of Minnesota losses due to preventable smuts. It is quite clear that many farmers do not treat the seed with copper sulphate or formaldehyde on account of the labor involved and the danger of injury to the seed. It is imperative, therefore, that simpler and safer methods be devised.

Table I

Minnesota losses due to smuts amenable to treatment by surface disinfection

	Loss in dollars			
	1917	1918	1919	1920
Wheat covered smut	2,083,000	5,690,000	453,940	189,225
Barley covered smut	317,000	350,400	211,120	232,000
Oat smuts	2,343,000	3,341,000	1,173,760	783,040
Total loss . . .	4,743,000	9,381,400	1,838,820	1,204,265
Average	$ 4,291,871			

The investigations summarized in this paper were outlined as a preliminary study to compare the efficiency of chemical dusts with that of formaldehyde in effectively controlling certain cereal smuts. The studies are grouped under three general headings: (1) seed germination tests, (2) effect of the fungicides onthe smut spores, and (3) the comparison of the effect of different methods of seed treatment on percentages of smut in field plots.

EXPERIMENTAL METHODS

The seed germination studies were carried out with wheat, oats, barley, and rye, principally in moist sand and on blotting paper. In all tests at least two hundred seeds were germinated from each separate lot. No attempt was made to control the moisture content or the temperature of the sand or soil in which the seed germinated. The seed which germinated in sand was simply sown in rows across flats of sand and watered according to the routine greenhouse schedule. Each series of rows was replicated in the flats to obtain as uniform conditions as possible for the growth of the plants in all rows. Care was taken also to plant all of the directly comparable seed lots in each germination series on the same day.

With the exception of the field tests the different grains were treated under laboratory conditions in small lots. The charts listed below show the proportions used.

To treat grain with a dust at the rate of 4 oz. per bushel, the following amounts were used:

Wheat	0.4 gm. dust to 100 gm. seed
Rye	0.4 gm. dust to 100 gm. seed
Oats	0.4 gm. dust to 50 gm. seed
Barley	0.4 gm. dust to 75 gm. seed

To treat grain with a concentrated formaldehyde spray (one part formaldehyde to one part water) at the rate of one quart to fifty bushels, the following amounts were used:

Wheat	0.154 cc. formaldehyde (1:1) to 200 gm. seed
Rye	0.154 cc. formaldehyde (1:1) to 200 gm. seed
Oats	0.154 cc. formaldehyde (1:1) to 100 gm. seed
Barley	0.154 cc. formaldehyde (1:1) to 150 gm. seed

After the grain was sprayed, it was placed in glass jars covered with blotting paper and left for five hours.

To treat grain by the standard sprinkle method, one pint of formaldehyde to forty gallons of water (1:340) for fifty bushels of grain, the following amounts were used:

Wheat	24 cc. formaldehyde (1:320) to 200 gm. seed
Rye	24 cc. formaldehyde (1:320) to 200 gm. seed
Oats	24 cc. formaldehyde (1:320) to 100 gm. seed
Barley	24 cc. formaldehyde (1:320) to 150 gm. seed

After the grain was sprinkled, it was covered for five hours in the same manner as is noted above.

All spore germination tests were made in Syracuse watch crystals which previously had been washed thoroughly with hot soap suds and rinsed in distilled water. It was found that the spores of _Tilletia_ _foetens_ and _Ustilago_ _levis_ germinated about equally well in tap water, distilled water and two percent sugar solutions at 22° C and 13° C. At 22° C, however, contaminating fungi grew rapidly in tap water and in the sugar solution, and it was difficult to make accurate counts of the spores which had germinated under these conditions. All subsequent germination tests were made, therefore, at 13° C and, whenever possible, in distilled water.

In preparing the moist sand for germinating spores it was found more convenient to pour dry sand into the Syracuse dishes first and then to add the water from a graduated pipet. This made it possible to get a smoother layer of sand in the dish than could have been obtained had the water been added to the sand before it was placed in the dish. To get uniformity in the moisture content of the material in each dish a measured amount of sand (12 grams) was used in each case and it was moistened with a definite amount of water (2 cc.) . Since the amount of water necessary to saturate the sand depends upon the size of the component particles, the dry sand always was sifted through screens of the same mesh. An intermediate grade of sand was used in all experiments.

Field tests to determine the effect of different treatments on the percentages of smut produced in oats and wheat were carried out by treating lots of badly smutted grain with various materials and subsequently making a count of the number of smutted heads in each plot. In counting the percentage of bunt in the plots of wheat, every head in each row was cut in two with a pair of shears to make sure that all bunted heads would be observed.

MATERIALS EMPLOYED

Four kinds of grain were used in the seed germination tests: Marquis wheat, Iowa 103 oats, Manchuria barley, and a spring rye (Minn. No. 61). In selecting the seeds for the germination tests care was taken to discard all kernels with badly injured seed coats. After three months of preliminary tests, it was decided to confine the spore germination experiments to two species, Ustilago levis (K. and S.) Mag., which causes the covered smut of oats, and Tilletia foetens (B. and C.) Trel., which causes the high bunt of wheat. A large percentage of the spores of U. levis always germinated in distilled water and thus lent themselves readily to any sort of test. The bunt spores, however, at first germinated capriciously, but it was soon discovered that the spores from a single smutted head germinated much more uniformly; and advantage was taken of this fact in the subsequent experiments. Three spores balls from each of twenty-five heads were broken and the spores were sown in distilled water. The spores from a few of these heads were much more viable than the spores of the other heads. These heads were therefore used as a source of smut balls for all of the Tilletia spore germination tests. It is also probable that spores from one smut ball germinate more uniformly than those from another ball, even if both smut balls are taken from the same head.

Seven fungicides were used in the tests: two formaldehyde solutions, formaldehyde gas, seed- o-san dust, chlorophol dust, copper carbonate dust, and Mackie's dust. Formaldehyde solutions were used in the two concentrations as recommended for the standard smut treatments; (1) the concentrated solution, which is one part 40 percent formaldehyde solution to one part water, and (2) the dilute solution, which is one part formaldehyde to three hundred and twenty parts of water (or one pint to forty gallons). The formaldehyde gas used was the fumes over a concentrated formaldehyde solution in a small sealed bottle.

"Seed -o- San" and "Chlorophol" are commercial dusts reported by the Standard Tester Company to be "fifty times as toxic to microorganisms as any other known germicides". After an effort to determine in general the chemical nature of these dusts, Dr. R. A. Gortner of the Division of Agricultural Chemistry, writes as follows: "The Chlorophol contains eighty-one percent ash, which is almost entirely sodium. No metals other than sodium and potassium were present. It gave a strong test for phenols and for organic chlorine, and I would say that in all probability it is a sodium salt of chlorinated phenol. Seed-o-San contains approximately forty-six percent ash, largely sodium carbonate. Only sodium and potassium are identified as metals. We have not been able to identify what the organic matter is, but both act by virtue of the organic matter, for both mercury and copper are entirely absent."

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Seven fungicides were used in the tests: two formaldehyde solutions, formaldehyde fumes, Seed-o-San dust, chlorophol dust, copper carbonate dust, and Mackie's dust. Formaldehyde solutions were used in the two concentrations recommended for the standard smut treatments; (1) the concentrated solution (one part of 40 percent formaldehyde to one part of water), and (2) the dilute solution (one part of formaldehyde to three hundred and twenty parts of water). The formaldehyde fumes were the fumes over a concentrated formaldehyde solution in a small sealed bottle.

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Two grades of copper carbonate were used, the chemically pure which has a copper equivalent of about fifty-two percent, and a commercial product obtained from the Corona Chemical Division of the Pittsburg Plate Glass Company which they call New Bordeaux Mixture. This dust has a copper equivalent of approximately twenty percent.

Mackie (11) used in his tests in California a dust made up of equal parts of dehydrated copper sulphate and calcium carbonate, and this mixture is named here for convenience, "Mackie's dust."

SEED GERMINATION STUDIES

A series of seed germination studies were outlined to determine and compare the amount of seed injury produced by different methods of treating grain. It seemed desirable (1) to compare the effect of formaldehyde and that of copper carbonate on the germination of cereal seeds, (2) to determine the effect on the seed germination of treating grain with different amounts of copper carbonate dust, (3) to test the effect on germination of storing seed wheat which had been dusted with copper carbonate, (4) to compare the effect of different chemical dusts on the percentage of germination of wheat, barley, oats, and rye, and (5) by the germination of a single seed lot series in three different ways, to obtain a basis for the comparison of all of the germination tests.

Germination tests to determine the effect of treating seed with copper carbonate dust have, in the past, been confined entirely to wheat. The effect of copper carbonate dust on the viability of wheat, oats, barley and rye was studied, therefore, in the present investigation.

Table II

The comparative effect of formaldehyde and copper carbonate dust on the germination of wheat, barley, oats, and rye.

Cereal treated	Treatment and percentage of germination			
	Formaldehyde sprinkle	Formaldehyde spray	Copper Carbonate dust	Check
Wheat (Marquis)	70	58	80	63
Oats (Iowa 108)	92	83	92	95
Barley (Manchuria)	96	96	96	97
Rye (Spring	63	23	74	60
Average	80	65	83	79

All percentages of germination are based on counts of two hundred seeds. The seeds were germinated in sandy loam under apparently uniform conditions.

Table II summarizes the results obtained from the germination of wheat, oats, barley, and rye which had been previously divided into four lots; the first was treated with formaldehyde, the third with copper carbonate dust, and the fourth left untreated. As a difference of ten percent in any of these tests might easily be attributed to the effect of random sampling, it seems that the only injury occurred when rye was treated with the formaldehyde spray. It will be noted that the seed lots treated with copper carbonate dust and those treated by the formaldehyde sprinkle method germinated better than the check lots.

Darnell-Smith and Ross (4) in Australia, and Mackie and Briggs (11) in California obtained a dust film on the seed coats of wheat by the use of two ounces of dust per bushel. However, under farm conditions it is doubtful whether the use of two ounces of dust per bushel will insure the effective treatment of every kernel in the sample dusted. There also may be, as indicated in Plate V, a tendency toward producing a higher yield with the use of a larger amount of copper carbonate dust per bushel. In considering the recommendation of the use of a larger proportion of dust, the question arose as to how much dust could be applied to the grain without producing injurious effects.

Table III

The effect on germination of adding different amounts of copper carbonate dust to wheat seed

Rate of Dusting	Percentage of germination on sandy loam after thirteen days	
	3 replicated seed lots	Average
2 oz. per bu.	92 83 80	85
4 oz. per bu.	85 81 90	85
6 oz. per bu.	98 92 78	89
10 oz. per bu.	98 90 80	89
no treatment	78 80 80	79

Table III shows the results obtained from the germination of seed lots dusted with varying amounts of copper carbonate to determine if injury would result from excessive dusting. It is apparent that the use of as much as ten ounces per bushel did not injure the seed. It evidently is safe, therefore, to add as much dust as is necessary to cover the seed thoroughly.

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An experiment was made to ascertain the effect of storing seed which had been dusted with copper carbonate. The results are summarized in Table IV.

Table IV

The effect on germination of storing wheat treated with copper carbonate dust

Amount of Dust	Date of Germination Test		
	Sept. 29, 1921	March 7, 1922	March 7, 1922
	Percentage of Germination		
	On blotting paper		On moist sand
2 oz. per bu.	92	86	51.5
Untreated	93	86.5	52

The first germination tests were made on blotting paper in September 1921. In March 1922 germination tests were again made, both on blotting paper and in moist sand. The percentage of germination on blotting paper in the last test was lower than that in the first test, but as the percentages in both the check and the treated portion were lowered, it is safe to conclude that the dust did not reduce the viability of the seed. The view was borne out further by the percentages of germination in moist sand. In this material the wheat germinated in March was also less viable than that germinated in September, but the treated seed again germinated equally as well as the check.

In addition to copper carbonate, two other chemical dusts, seed-o-san and Mackie's dust, were considered valuable as smut preventives. They were therefore used in treating seed to determine their comparative effect on the viability of the seeds of different cereals. The results of this experiment are summarized in Table V.

Table V

A comparison of the effect of four chemical dusts on the percentage of germination of wheat, barley, oats, and rye

Treatment	Percentage of Germination[1]							
	Wheat		Oats		Barley		Rye	
	Replicated tests	Av.	Replicated tests	Av.	Replicated tests	Av.	Replicated tests	Av.
Seed-o-San	92 93 87	91	96 98 88	93	95 98 98	97	84 85 92	87
Mackie's Dust	57 65 55	59	100 98 100	99	86 92 94	91	78 76 82	79
Copper carbonate, 52% Cu equivalent	62 56 58	59	92 90 94	92	95 98 95	96	77 79 75	77
Copper carbonate, 20% Cu equivalent	53 72 65	63	98 98 100	99	98 94 95	96	79 82 73	78
Check	68 61 58	62	96 97 97	97	96 97 93	95	86 78 78	81

[1] Three separate tests were made and the results are given in order

It is evident from an examination of the germination percentages of the check plots that the percentages of the comparable replicated tests with wheat or rye vary much more than do those with oats or barley. This same phenomenon is apparent regardless of the treatment used. As wheat and rye are also more liable to injury from formaldehyde, a natural explanation presents itself in the fact that the lemma and palea adhering to the oats and barley seeds afford them extra protection.

The Seed-o-San dust stimulated the germination of wheat seed very considerably when it was planted in moist sand. This was consistently true in all of the tests. However, only the percentage of germination was increased. The untreated seed and that treated with the other chemicals germinated just as quickly and the seedlings grew as vigorously, but not as many seeds germinated. The Seed-o-San did not increase the percentage of germination of oats, barley or rye. This may have been due to the fact that there were more seeds of low vitality in the wheat seed than in the seed lots of the other cereals.

The results were so striking that the tests were repeated in sandy loam and in order to determine whether the stimulating effect of Seed-o-San on wheat would be equally as pronounced in sandy loam as in clean sand. Germination tests also were made on blotting paper. The results are summarized in Table VI.

Table VI

A comparison of germination tests on blotting paper, in sandy loam, and in clean sand, of a single lot of wheat treated with four chemical dusts.

Treatment[2]	Percentage of Germination					
	In sandy loam[1]		In clean sand		On blotting paper	
	Replicated tests	Av.	Replicated tests	Av.	Replicated tests	Av.
Seed-o-San	87[3] 86 83	85	92 93 87	91	96 99	97.5
Mackie's Dust	37 46 57	44	57 65 55	59	97 95	96
Copper carbonate dust - 52% Cu equivalent	47 55 54	52	62 56 58	59	95 96	95.5
Copper carbonate dust - 20% Cu equivalent	54 49 54	52	53 72 65	63	94 92	93
Untreated	48 39 59	45	68 61 58	62	96 99	97.5

[1]Three parts clay loam to two parts clean sand [2]Marquis wheat used thruout
[3]Seedlings in these lots were all distinctly more vigorous than the other lots in this test, averaging an inch taller than the other seedlings.

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It is quite apparent from Table VI that Seed-o-San again increased the percentage of germination of wheat seed sown in clean sand. The percentage of germination was increased also in sandy loam. But in this soil the seedlings were, in addition, much more vigorous than were those which developed from the other seed lots. At the end of a week after they had emerged from the soil the seedlings in the Seed-o-San plats were a half inch taller than those in any of the other plats. (See Plate I.) This compound stimulated germination of seed and increased the vigor of seedlings so strikingly that it almost seems as though it would be valuable, under certain conditions, to treat seed with this material regardless of its fungicidal value, which has not yet been sufficiently tested. As will be shown later, Seed-o-San did not inhibit the germination of spores of *Ustilago levis* or *Tilletia foetens*. (See Table XIII and Plate II) All seed germinated almost equally well on blotting paper.

SPORE GERMINATION STUDIES

Two series of germination tests were outlined to determine the effect of formaldehyde on bunt spores inside of treated smut balls. However, the principal studies were devised to ascertain the effect of certain germicidal dusts, especially copper carbonate, on the spores of *Ustilago levis* and *Tilletia foetens* under conditions approximating those attained when the treated grain seeds germinate in soil in the presence of smut spores.

Practically all investigators agree that formaldehyde used as recommended for the standard treatments will kill any smut spores lodged on the outside of the seed coats. It is generally believed, however, that spores inside a smut ball are able, under certain conditions, to withstand formaldehyde treatments. This is indicated by the recommendation given by most pathologists that, to obtain the best results in treating wheat with formalde-

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hyde, all smut balls should be fanned out before the grain is treated.

Tests were made to determine whether bunt spores inside of spore balls would be killed by exposing the spore balls to formaldehyde gas for varying lengths of time.

Table VII

The effect of formaldehyde gas on smut spores inside smut balls.

Length of time[1] exposed to formaldehyde fumes	Percentage of Germination in distilled water	
	After 4 days	After 7 days
1 hour	2	50
2 hours	50	50
3 hours	2	50
4 hours	2	50
6 hours	0	0.5
24 hours	0	0[2]
Untreated smut balls	0	50

[1]The spore balls were suspended by means of a paraffin coated basket, over a 1:1 solution of formaldehyde in a small tightly corked bottle and the spores were germinated in distilled water at 13° C immediately after treating.

[2]The contents of seven spore balls were tested in separate trials after twenty-four hours exposure to formaldehyde gas and in all cases the spores failed to germinate.

Table VII summarizes the results obtained from these tests in formaldehyde gas. The spore balls were suspended in formaldehyde fumes for the lengths of time indicated in the first column of the table. Spores in the smut balls subjected to the fumes for less than four hours remained uninjured, while all of the spores were killed in the balls treated for twenty-four

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hours. It would seem, therefore, that the standard formaldehyde treatments must rely either on the action of formaldehyde in solution or on the residual effect of para formaldehyde for the killing of the spores inside of smut balls.

This naturally raised the question as to what effect the soaking of smut balls in a formaldehyde solution would have on the spores inside. The results of soaking smut balls in a solution of formaldehyde (1 part formaldehyde to 32 parts of water) for varying lengths of time are summarized in Table VIII.

Table VIII

The effect on the viability of spores of soaking spore balls for varying lengths of time in a solution (1:320) of formaldehyde

Time in Solution	Percentage of Germination											
	From smutted head #2 moist conditions[1]						From smutted head #3 dry conditions[2]					
	Test 1			Test 2			Test 3			Test 4		
After:	2dys	5dys	6dys	2dys	5dys	6dys	2dys	5dys	6dys	2dys	5dys	6dys
1 minute	0	0	0	0	0	0	0	0	0	0	0	0
3 minutes	0	0	0	0	.1	.1	0	0	0	0	0	0
7 "	0	50	70	0	50	80	0	.5	.5	0	.5	.5
10 "	0	60	60	0	60	60	0	25	25	0	10	25
12 "	0	0	0	0	25	50	0	0	0	0	0	0
Check, no treatment to compare head #2 with head #3	0	60	80	0	60	80	0	10	10	0	10	10

[1] After the smut balls were removed from the formaldehyde solution, they were placed on wet blotting paper under a small bell jar for twelve hours. The balls, then thoroughly wet, were broken open and their contents sown on distilled water to germinate.

[2] After these smut balls were removed from the formaldehyde solution they were placed on dry blotting paper for twelve hours. The contents of the dried balls were then germinated in distilled water.

These results showed uniformly, in four separate tests, that the spores inside the spore balls were unable to germinate normally after the spore balls had been soaked in the solution for from one to three minutes. Spores from those balls which had remained from seven to ten minutes in the formaldehyde solution, however, germinated readily. In explaining the results obtained in these tests, it is hardly probable that the spores inside these smut balls had been first weakened by the action of the formaldehyde and then subsequently revived. However, it is possible that para formaldehyde was formed in the spore balls soaked for the shorter lengths of time and not in those soaked for ten minutes. This view is supported by the fact that spores from smut balls which were soaked for three minutes and then dried rapidly were injured as much as were the spores from smut balls which were soaked for seven minutes and then kept moist until germination tests were made.

There would seem, therefore, to be an indication that more para formaldehyde was formed in the spore balls not so thoroughly wet, due to a quicker rate of drying out. The residual action of this para formaldehyde would probably be analogous to that pointed out by Hurd (8) in the production of seed injury. She observed the formation of para formaldehyde on the seed coats of grain which had been dried out quickly after dipping in a formaldehyde solution and gave the following explanation for its action, "Paraformaldehyde is very unstable and is constantly breaking down into formaldehyde gas. Thus the seed, unless well spread, is surrounded by this toxic vapor which penetrates the seed coat, probably again going into solution in the presence of any moisture in the seed covering."

In considering the effect of chemically dusted grain on smut spores in the soil around the seeds, the question naturally arises as to how far around a dusted kernel of grain are the spores affected by the presence of the chemical dust. To answer this question, experiments were devised in which spores of Tilletia foetens and Ustilago levis were dusted over moist sand into which seeds of different lots which had been each treated with different chemical dusts were partially buried. After four or five days spores were washed off from particles of sand which were taken at different distances from the treated seed. The spores were then examined under the microscope to determine the percentage of germination which had taken place at the distances indicated in Tables IX and X.

Table IX

The effect of chemically dusted grain on spores of *Ustilago levis* germinating around the seed in moist sand.

Kind of Dust	Percentage of Germination							
	1 cm. from treated seed		.5 cm. from treated seed		Edge of treated seed		Under treated seed	
	3 dys.	4 dys.	3 dys.	4 dys.	3 dys.	4 dys.	3 dys.	4 dys.
Copper carbonate dust	0.5	35	0.5	35	0.5	25	0.5	10
Mackie's Dust	50	35	15	45	15	25	1	35
Chlorophol	50	35	30	35	10	10	1	10
Check	0	30	20	30	15	5	15	5

Table X

The effect of a chemically dusted wheat kernel on Tilletia foetens spores germinating around it in moist sand.

((Spores from Head #3 (Series #8))

Kind of Dust	Percentage of Germination			
	1 cm. from treated seed	.5 cm. from treated seed	At edge of treated seed	Under treated seed
Copper carbonate dust	2 - 5	2 - 5	0.5	.05 *
Copper sulphate &lime	15 -20	2 - 5	2 - 5	.05 x
Chlorophol dust	15 -20	15 -20	.1 x	.5 x
Check	15- 20	15 -20	10 -15	10 - 15

* No sporidia   x Sporidia very rare

The tests show that neither copper carbonate dust, Mackie's Dust nor chlorophol had any apparent effect on the percentage of germination of the spores of Ustilago levis even directly underneath the treated seed. However, copper carbonate seemed to reduce the percentage of germination of spores of Tilletia foetens at a distance of one centimeter from the seed. Mackie's Dust reduced the germination to a distance of half a centimeter from the treated seed, and the effect of chlorophol was noticeable only at the edge of the seeds.

In this test it was impossible to pick up spores from the seed coat without including some from surrounding sand grains, therefore another series of germination tests was outlined to determine the extent to which spores actually resting on the seed coat were injured by the chemical dusts. In these tests spores were dusted only onto the surface of the treated kernels. In each Syracuse watch crystal, containing moist sand, four seeds were placed. When using Tilletia foetens spore material, all four of these seeds were dusted with spores from a single spore ball. Two of them, one treated and the other left untreated as a check, were buried in the sand while the other two were only partially covered. The results of these tests are summarized in Table XI.

Table XI

The effect of chemical dusts on the germination of smut spores placed on treated seed in moist sand.

Kind of Dust	Percentage of Germination			
	Ustilago levis		Tilletia foetens	
	Seed covered	Seed partially covered	Seed covered	Seed partially covered
Copper carbonate, 52% Cu equivalent	20[1]	25[1]	2	4
Check	25	25	1	20
Copper carbonate, 20% Cu equivalent	20	25	1 [2]	3 [2]
Check	15	30	40	25
Seed-o-San	50	15	8	2
Check	50	70	8	5
Mackie's Dust	10	10	15	20
Check	10	15	15	20

[1] In these cases the spores germinated in as great abundance as the checks, but the germ tubes were distinctly shorter than those of the spores in the check lots.

[2] The differences between the spore germination on these treated seeds and that on their respective checks was apparent macroscopically. (See text.)

The copper carbonate treatment seemed to stunt the germ tubes of Ustilago levis although the percentage of germination in all cases was apparently as great as in the checks. Only the copper carbonate dusts injured the spores of Tilletia foetens distinctly. Copper carbonate, twenty percent equivalent, so distinctly injured the spores on the seed coats that the difference between the treated and untreated seeds was apparent macroscopically, the check seeds became white and fuzzy with a covering of germinating spores while the treated seeds remained clean.

To test further the differences in the effects of the chemical dusts on the two kinds of smut spores, a series of spore germination trials were made on distilled water containing treated seeds. Table XII indicates the results obtained from the germination of the spores of Ustilago levis.

Table XII

The effect of traces of chemical dust on the germination of spores of Ustilago levis

Treatment	Percentage of Germination			
	1 treated seed in 5 cc. water		3 treated seeds in 5 cc. water	
	After 3 days	After 5 days	After 3 days	After 5 days
Seed-o-San	85	85	85	85
Copper carbonate, 52% Cu equivalent	15	20	5	5
Copper carbonate, 20% Cu equivalent	30	20	5	3
Mackie's Dust	55	50	30	5
Untreated Seed	55	60	82	85
Distilled Water	80	80	50	60

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No significant differences were noted in the trials in which one treated seed was used in the five cubic centimeters of water, but when using the copper dusts the presence of three treated seeds in the water distinctly lowered the percentage of spore germination and produced a stunting of the germ tubes (shown in Plate II). The presence of Seed-o-San dust seemed to have no effect on the germination of the spores.

In similar trials, using Tilletia foetens spores, the effect of the treated seeds was even more marked. (See Table XIII)

Table XIII

The effect of traces of chemical dust on the germination of spores of Tilletia foetens

Treatment	Percentage of Germination			
	1 treated seed in 5 cc. water		3 treated seeds in 5 cc. water	
	after 5 days	after 6 days	after 5 days	after 6 days
Seed-o-San	60	75	10	70
Copper carbonate, 52% Cu equivalent	0	0	0	0
Copper carbonate, 20% Cu equivalent	0	0	0	0
Mackie's Dust	0	0	0	0
Untreated Seed	15	15	85	85
Distilled Water	20	25	75	90

Germination of the wheat spores was completely checked by the presence of the copper dusts, while Seed-o-San again had no effect on the spore germination.

As the Seed-o-San failed to injure the smut spores in any of the trials, its fungicidal value is doubtful. Therefore if Seed-o-San controls smut of oats or wheat smut in the field, the control would probably be due to seed stimulation and not to spore injury.

FIELD EXPERIMENTS

In the spring of 1921 field experiments were begun to determine the effect of treating smutty seed of wheat and of oats with formaldehyde and with copper carbonate dust. Badly smutted seed lots of Prelude wheat and of Victory oats were obtained. The methods of treatment and the results are given in Tables XIV and XV, and the differences in plot yields are illustrated in Plates IV, V, VI, and VII.

Copper carbonate dust and both of the formaldehyde treatments practically eliminated bunt from the wheat and both the loose and covered smuts of oats, although copper carbonate was more effective than formaldehyde. However, the treatments with formaldehyde reduced the yield, especially when the seed was treated several weeks before sowing. It is very evident that it is unsafe to use the concentrated formaldehyde method on wheat, although it is less injurious to oats. When wheat was sprayed with a 50:50 formaldehyde solution some time before planting the seed was injured so severely that practically none germinated. The copper carbonate dust, on the other hand, increased the yield. Furthermore, this increase was somewhat proportional to the amount of dust used. It seems perfectly clear that the copper carbonate dust treatment was far superior to formaldehyde under the conditions of this experiment.

Table XIV

Comparative field studies with seed treated for the control of bunt in wheat

Treatment	Heads Counted	Percent Germination*	Yield bu. per acre	Percent Smut
Treated with (50:50) formaldehyde immediately before planting. Smut balls not fanned out.	500	44	11	3
Treated with (50:50) formaldehyde immediately before planting. Smut balls fanned out.	500	53	13.3	0
Treated with (50:50) formaldehyde three weeks before planting. Smut balls not fanned out.	200	23	3.3	3.5
Treated with (50:50) formaldehyde three weeks before planting. Smut balls fanned out.	2 plants	26	0	0
Treated with (1:320) formaldehyde immediately before planting. Smut balls not fanned out.	500	58	7	0
Treated with (1:320) formaldehyde three weeks before planting. Smut balls fanned out.	500	44	10	1
Treated with (1:320) formaldehyde immediately before planting. Smut balls not fanned out.	500	52	12.3	0.4
Treated with (1:320) formaldehyde three weeks before planting. Smut balls fanned out.	500	65	10	0
Treated with 4 oz. copper carbonate dust per bushel. Smut balls not fanned out.	500	70	19.3	0
Treated with 4 oz. copper carbonate dust per bushel. Smut balls fanned out.	500	72	20	0
Treated with 2 oz. copper carbonate dust per bushel. Smut balls fanned out.	500	78	14.6	0

* Germination tests were made on blotting paper.

Table XIV (Continued)

Treatment	Heads Counted	Percent Germination *	Yield bu. per acre	Percent Smut
Washed in water immediately before planting. Smut balls fanned out.	500	0	14.6	4
Check Smut balls not fanned out.	500	0	14.6	21
Check Smut balls fanned out.	500	86	15.3	16

* Germination tests were made on blotting paper.

Table XV

Comparative field studies with seed treated for the control of smut of oats

Treatment	Heads Counted	Percent Germination *	Yield bu. per acre	Percent Smut
Treated with (50:50) formaldehyde, immediately before planting.	500	95	92.9	3
Treated with (1:320) formaldehyde, immediately before planting.	500	93	91.7	0.2
Treated with copper carbonate dust immediately before planting.	500	89	85.8	0
Check	500	98	64.6	13

* Germination tests were made on blotting paper.

DISCUSSION AND CONCLUSIONS

It is quite evident that both the formaldehyde and copper sulphate methods of seed treatment are effective in smut control. Either method, however, is more or less cumbersome and often results in impaired germination of the seed. Seed treated with liquids swell considerably and are likely to stick in the drills. Furthermore, in regions where it is necessary to sow grain early, the wet seed is liable to injury from freezing. In the Palouse district of the northwest, where the soil is practically always dry at the time of sowing, formaldehyde cannot be used because of the poor germination resulting from seed treated with formaldehyde and planted in dry soil. Copper sulphate is the chief disinfectant used in this region and it also may injure the seed quite appreciably under some conditions. Grain with broken seed coats is especially susceptible to injury from copper sulphate.

Numerous attempts have been made in the past twenty years to modify the above treatments with the view of eliminating the injury to the seed. Some success has been obtained, but the modifications have nearly always made the treatment still more cumbersome. Any increase in the difficulty of application of a treatment must be avoided because the labor involved is one of the most important factors determining its practicability.

The average annual loss of $4,291,871 for the past four years in Minnesota, due to preventable smuts, shows clearly that comparatively few Minnesota farmers treat their seed grain for smut.

The concentrated formaldehyde method was devised and recommended as a simplified method of treating oats but has proven unsafe for wheat.

The dry copper carbonate method seems to meet most of the objections raised against the formaldehyde and copper sulphate methods. It is efficient in controlling smuts, does not cause seed injury and is easy to apply. The advantages of this method over the liquid methods may be listed as follows:

1. No water is required and hence there is no danger from freezing.
2. The danger of seed injury is eliminated.
3. Grain can be treated a long while before it is sown.
4. The process of treating is fool proof.
5. The dry treatment involves less time and labor than the wet methods.

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SUMMARY

1. Treatment of wheat with from two to ten ounces of copper carbonate per bushel did not reduce the viability of the seed when planted in soil in the greenhouse. A limited number of experiments indicate that barley, oats and rye also may not be injured by the same treatment.

2. Wheat dusted with copper carbonate and stored for six months and then germinated showed no loss in vitality due to the presence of the chemical dust.

3. Seed treated with Seed-o-San dust, a proprietary preparation for the prevention of cereal smuts, germinated better than untreated seed or seed treated with the other chemical dusts. However, this same Seed-o-San dust, under the conditions of the experiments, had no apparent effect on the germination of the spores of either *Tilletia foetens* or *Ustilago levis*.

4. A comparison of the germination of several seed lots on moist sand, sandy loam and blotting paper showed that the results obtained may vary greatly with the different substrata. It is important, therefore, when comparing results of germination tests, to know the substratum used.

5. Exposing spore balls of *Tilletia foetens* to formaldehyde fumes for from one to four hours did not impair the viability of the spores inside the balls. However, all of the spores inside of the balls were killed by exposure for twenty-four hours.

6. Most of the spores within smut balls were killed by soaking the balls in a solution of formaldehyde (1-320) for from one to three minutes and allowing them to stand for twelve hours after removal from the solution. However, if the smut balls were soaked for from seven to ten minutes in the

solution and then dried over night, there was much less spore injury. A possible explanation for this is offered.

7. The presence of seeds treated with copper carbonate or Mackie's dust in moist sand lowered the percentage of germination of spores of Tilletia foetens. This influence extended to a distance of from 0.5 cm. to 1 cm. from the treated seed. The spores of Ustilago levis, however, germinated well directly beneath the treated seed.

8. Spores of Ustilago levis on the surface of seeds treated with copper carbonate and Mackie's dust germinated abundantly in moist sand, but the germ tubes were stunted. Similar results were obtained when the spores were germinated in water containing traces of these copper dusts.

9. The treatment of seed with copper carbonate efficiently controlled the covered smut of wheat and the covered smut of oats.

10. In field experiments seed treated with four ounces of copper carbonate per bushel yielded 4.7 bushels per acre more than the checks. Seed treated by each of the other methods yielded less than the checks.

11. There is not sufficient evidence to warrant the recommendation of any of the other dusts tested in these experiments.

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Plate I

The effect of germinating Ustilago levis spores in 5 cc. of water containing three seeds treated with chemical dusts. The letters on the chart indicate the kind of chemical used.

Observations made after three days:

A Copper carbonate (52 percent copper equivalent)

B Copper carbonate (20 percent copper equivalent)

C Mackie's Dust

D Seed-o-San

E Untreated seed

F Distilled water only

Observations made after five days:

AA Copper carbonate (52 percent copper equivalent)

BB Copper carbonate (20 percent copper equivalent)

CC Mackie's Dust

DD Seed-o-San

EE Untreated seed

FF Distilled water only

EFFECT OF CHEMICAL DUSTS ON SPORE GERMINATION OF USTILAGO LEVIS.

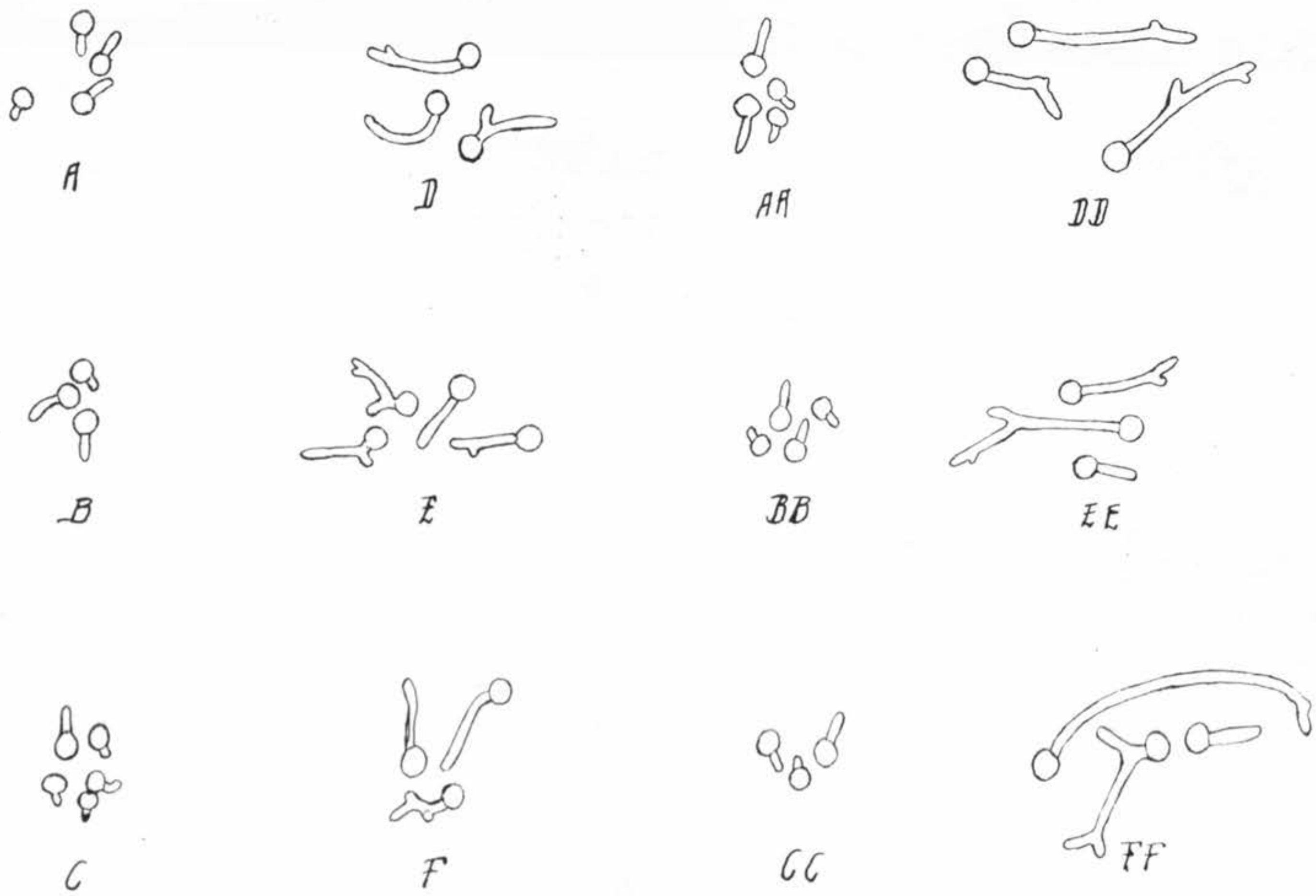

Plate I

Plate II

Seedling stand from seed lots treated with different dusts and grown in sandy loam. Observing the rows from left to right, the materials used for treating seed were: Seed-o-San, Mackie's Dust, Copper Carbonate 52 percent copper equivalent, Copper carbonate 20 percent copper equivalent, and no treatment.

Plate II

Plate III

Two kernels each of wheat, oats, barley, and rye. The seed on the left of each pair was dusted with dry copper carbonate and that on the right was not treated.

Plate III

Plate IV

Plot yield comparisons of wheat untreated with that from seed treated with a 50/50 concentrated formaldehyde spray; one pint to 50 bushels and covered 5 hours.

Check A — Wheat from untreated plot not fanned
Yield 14.6 bu. Smut 21%

Check AA — Wheat from untreated plot, smut balls fanned out
Yield 15.3 bu. Smut 16%

A Bundle — Wheat not fanned; treated immediately before planting
Yield 11 bu. Smut 3%

AA Bundle — Wheat fanned; treated immediately before planting
Yield 13.3 bu. Smut 0%

B Bundle — Wheat not fanned; treated three weeks before planting
Yield 3.3 bu. Smut 3.5%

BB Bundle — Wheat fanned; treated three weeks before planting
Only two plants

Plate IV

Plate V

Plot yield comparisons of wheat untreated with that from seed treated with a standard formaldehyde sprinkle; 1-320 and covered 5 hours.

Check A	Wheat from untreated plot not fanned	Yield 14.6 bu.	Smut 21%
Check AA	Wheat from untreated plot, smut balls fanned out	Yield 15.3 bu.	Smut 16%
A Bundle	Wheat not fanned; treated immediately before planting	Yield 7 bu.	Smut 0%
AA Bundle	Wheat fanned; treated immediately before planting	Yield 10 bu.	Smut 1%
B Bundle	Wheat not fanned; treated three weeks before planting	Yield 12.3 bu.	Smut .4%
BB Bundle	Wheat fanned; treated three weeks before planting	Yield 10 bu.	Smut 0%

Plate V

Plate VI

Plot yield comparisons of wheat untreated with that from seed treated with a copper carbonate dust; dusted with mechanical duster and thoroughly mixed.

Check A		Wheat from untreated plot not fanned Yield 14.6 bu. Smut 21%
Check AA		Wheat from untreated plot, smut balls fanned out. Yield 15.3 bu. Smut 16%
A	Bundle	Wheat not fanned; dusted 4 ounces per bushel; treated immediately before planting Yield 19.3 bu. Smut 0%
AA	Bundle	Wheat fanned; dusted 4 ounces per bushel; treated immediately before planting Yield 20 bu. Smut 0%
B	Bundle	Wheat fanned; dusted 2 ounces per bushel; treated immediately before planting. Yield 14.6 bu. Smut 0%

Plate VI

Plate VII

Plot yield comparisons of wheat untreated with that from seed washed in water for one hour.

Check A	Wheat from untreated plot not fanned	
	Yield 14.6 bu.	Smut 21%
Check AA	Wheat from untreated plot, smut balls fanned out	
	Yield 15.3 bu.	Smut 16%
AA Bundle	Wheat fanned; washed for one hour in water immediately before planting	
	Yield 14.6 bu.	Smut 4%

Plate VII